INPs (inhibitors of the type III secretion system) reduce virulence of *Pseudomonas aeruginosa* towards A549 alveolar epithelial cells

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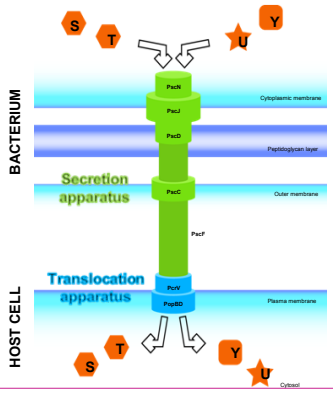
Type III secretion system (TTSS)



INTRODUCTION AND OBJECTIVES

Many pathogenic Gram-negative bacteria possess a type III secretion system (TTSS) that allows the secretion and translocation of effector proteins from the bacterial cytoplasm directly into the eukaryotic host cells. In *Pseudomonas aeruginosa* (*Pa*), the TTSS induces disruption of cytoskeleton and cytotoxicity leading to inflammation and tissue damage. A functional TTSS contributes to the establishment and dissemination of acute infections (1). Because of the alarming increase of resistance to current antibiotics and of the lack of novel drugs acting on multiresistant strains, new therapeutic approaches need to be explored, among which the inhibition of virulence mechanisms.

INPs are inhibitors of TTSS, which have shown activity against various bacterial species, including *Salmonella* spp, *Yersinia* spp, and *Chlamydia* spp (2,3). In this study, we evaluated the activity of these INPs against *Pa.* Our aim was to determine the effects on TTSS transcription and on the cytotoxicity of *Pa* towards alveolar epithelial cells.

METHODS

Strains: CHA (clinical isolate with high expression of TTSS) and the corresponding bioluminescent reporter strain CHA pC lux (constructed by transcriptional fusion of lux operon (luxCDABE) to the promoter of the regulator operon *exsCEBA*) (4).

Cells: human alveolar epithelial A549 cells.

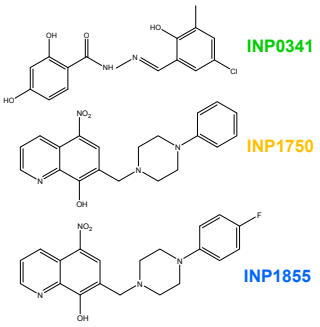
Cell viability: Effects on INPs on cell viability and protection towards *Pa.* cytotoxicity were assessed by measuring the release of the cytosolic enzyme lactate dehydrogenase (LDH) in the culture medium. Cells were incubated for 7 h in presence of increasing concentrations of INPs and/or CHA strain (10 bact./ cell).

TTSS transcription assay: Bioluminescence of the reporter strain was followed either over time for bacteria exposed to 60 µM of INPs (kinetics), or after 7 h of incubation with increasing concentrations of INPs (dose-response curve).

Growth inhibition assay: Bacterial cultures were incubated with INPs at increasing concentrations for 7h, after which aliquots were plated for enumeration of bacteria by colony counting.

RESULTS

Structure of INPs



Inocuity of INPs

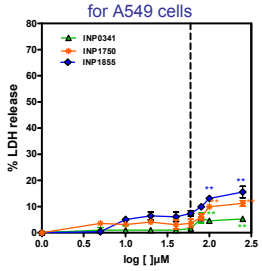


Figure 1 : percentage of LDH release induced by INPs after 7h of incubation. Mean ± sem (n=3). Dotted line : 60 µM

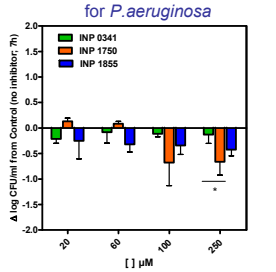


Figure 2 : difference in log CFU/ml recorded for bacteria incubated for 7h in the presence or in the absence of INP (DMEM with 10% FCS + DMSO). Mean± SD (*n*=3). * p<0.05

Correlation between inhibition of TTSS transcriptional activation and inhibition of TTSS-dependent cytotoxicity

Figure 5: Correlation between inhibition of regulator *exsCEBA* transcriptional activation and inhibition of CHA cytotoxicity towards A549 epithelial cells. Concentrations for which inhibition of toxicity decreased were not indicated.

Effects of INPs on *Pa* cytotoxicity

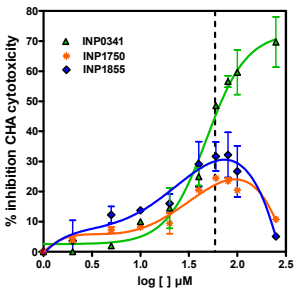


Figure 3 : percentage of inhibition of CHA cytotoxicity towards A549 epithelial cells by INPs after 7h of incubation. Mean ± sem (n=3). Dotted line : 60 µM

Effects of INPs on TTSS transcriptional activation

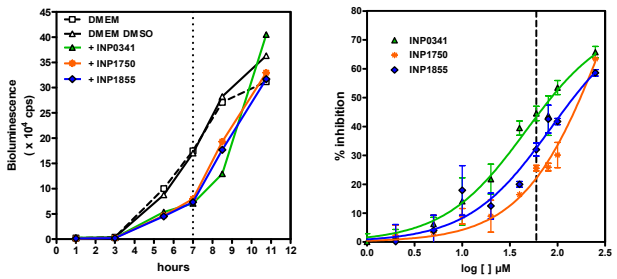


Figure 4 : (left panel) Reporter strains were incubated with 60µM of INPs for 12h. Luminescence (Counts per second CPS) was measured over time. Dotted line: time selected for further experiments.
(right panel) Dose-response curves on TTSS transcriptional activation of *exsCEBA* operon. Reporter strains were incubated with increasing concentrations of INPs for 7h. Dotted line: 60 µM. Values are expressed in percentage of inhibition of luminescence recorded in the absence of INPs. Values are means ± SD (n=3) .

Summary table:
[a] calculated from the equation of the sigmoidal regression of the corresponding dose-effect (using GraphPad prism). Statistical analysis: values compared to a negative control [INP406]:
* p<0.05, ** p< 0.01

TTSS inhibitors 60 µM	CHA cytotoxicity (% inhibition at 7 h) (A)	transcriptional activation of regulator *exsCEBA* (% inhibition at 7 h)[a] (B)	CHA growth (% inhibition at 7 h) (C)	INP cytotoxicity (% LDH release) (D)
INP0341	48.6 ± 0.9 **	43.3 **	1.2 ± 3.3	0 ± 0.9
INP1750	24.5 ± 0.3 **	22.1 **	0 ± 0.7	3.6 ± 1.6 *
INP1855	31.7 ± 5.7 **	32.6 **	4.9 ± 2.3 *	7.4 ± 0.3 **

- INPs at 60 µM did not affect bacterial growth **(Figure 2; C)** and induced minimal toxicity (<10%) for epithelial cells **(Figure 1; D)**
- INP0341, INP1750, and INP1855 decreased both the cytotoxicity of *P.aeruginosa* strain **(Figure 3; A)** and the transcriptional activation of the regulator operon *exsCEBA* **(Figure 4; B)**
- Dose–response experiments suggest that these effects were dependent on INP concentrations **(Figures 3 et 4)**
- From more potent to less potent: INP0341 > INP1855 > INP1750
- INP0341 may show a linear correlation between inhibition of transcriptional activation of TTSS regulator *exsCEBA* and inhibition of *P.aeruginosa* cytotoxicity **(Figure 5)**

CONCLUSIONS

INP0341, INP1750, and INP1855 protect A549 epithelial cells from *P.aeruginosa*-induced cytotoxicity in a way that seems related to their inhibitory effect on TTSS transcriptional activation but not to an inhibition of bacterial growth. Thus, this study suggests a potential interest for these TTSS inhibitors as anti-pseudomonal agents and warrants further studies aimed at better characterizing the differences between these INPs and their molecular mechanism of action

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

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