B-059

ICAAC 2014
Washington, DC | September 5-9

Targeting Type Three Secretion System in *P. aeruginosa* with the Inhibitor INP1855 Reduces Toxin and Inflammasome-Dependent Virulence *in vitro* and *in vivo*

A. Anantharajah [1], E. Faure [2,3], J. M. Buyck [1], Y. Glupczynski [4], C. Sundin [5], T. Lindmark [6], P. M. Tulkens [1], M-P. Mingeot-Leclercq [1], B. Guery [2,3], F. Van Bambeke [1]

[1] Pharmacologie cellulaire et moléculaire, Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium; [2] Lille-2 University hospital, Lille, France, [3]Host-Pathogen translational Research group Pseudomonas aeruginosa, Lille, France; [4] Laboratoire de microbiologie, CHU Mont-Godinne Dinant, Yvoir, Belgium; [5] Umea University, Umea, Sweden, [6] SwedenBio, Stockholm, Sweden

Mailing address:
Françoise Van Bambeke
av. Mounier 73, bt.73.05
1200 Brussels – Belgium
francoise.vanbambeke@uclouvain.be

ABSTRACT (revised)

Background: T3SS is associated with poor clinical outcome in P.a. acute infections. T3SS allows toxin injection in host cell cytosol. In phagocytes, it causes caspase-1 proteolysis via NLCR4 inflammasome activation. Caspase-1 induces cell death and secretion of IL-1β and IL-18 inflammatory cytokines. The latter repress IL-17 response, thereby impairing P.a. clearance (PMID 24555512). We studied the effect of the T3SS inhibitor INP1855 (PMID 22525317) on the cytotoxicity of lab. strains and clinical isolates of P.a. towards epithelial and phagocytic cells in vitro and its efficacy in vivo.

Methods: Strains: see Table; Cells: and A549 lung epithelial cells and THP-1 monocytes; Mice: C57BL6. T3SS transcription: Real-time PCR. Cytotoxicity: release of lactate dehydrogenase (LDH) in culture medium. Mouse model: acute lung injury induced by CHA (T3SS+) or CHAΔpopBD (T3SS-) with or without co-instillation of INP1855. Cytokines measured by ELISA in bronchoalveolar lavage (BAL).

Results: In vitro, ExoU+ strains were highly toxic for both cell types. For ExoU- strains, toxicity was related to the level of ExoS expression for A549 cells but rather to the expression of the T3SS translocation apparatus for THP-1 cells (via inflammasome activation; ECCMID2014 P0174). INP1855 decreased toxicity of T3SS+ strains, whether mediated by toxins or by translocation apparatus. In vivo, INP1855 decreased mortality and bacterial burden only in mice infected by T3SS+ CHA strain, with reduction of IL-1β and IL-18 and increase of IL-17 secretion in BAL.

Conclusion: Targeting T3SS apparatus decreases P.a toxicity by preventing toxin effects and inflammasome activation in phagocytic cells, which improves mice survival and bacterial clearance.

REFERENCES

[1] Hauser AR *et al* Nat Rev Microbiol 2009;7:654-665
[2] Miao EA and Warren SE, Journal of Clinical Immunology 2010; 30(4): 502-506
[3] Cohen TS and Prince AS, Journal of Clinical Investigation 2013; 23(4):1630-1637
[4] Faure *et al*., Am J Respir Crit Care Med. 2014;189(7):799-811
[5] Enquist, Bioorg Med Chem Lett. 2012; 22: 3550–3553

ACKOWLEDGMENTS

We thank K. Santos for excellent technical assistance. We thank Pr. Toussaint (Grenoble, France) for the kind gift of CHA. A. Anantharajah is doctoral fellow of the Belgian *Fonds de la recherche scientifique (F.S.R-FNRS).* This work was supported by the Belgian *Fonds de la Recherche Scientifique* and the Interuniversity Attraction Poles Programme of the Belgian Science Policy Office.

INTRODUCTION

Expression of Type III secretion system (T3SS) in *P.aeruginosa* (P.a) is associated with poor clinical outcome and high morbidity in acute infections. T3SS allows bacteria to inject exotoxins (e.g. ExoU or ExoS) into the host cell cytoplasm, causing cytotoxicity and preventing P.a. internalization [1]. In phagocytic cells, T3SS can also deliver flagellin FliC or T3SS rod proteins into the mammalian cytosol, inducing caspase-1 proteolysis via NLRC4 inflammasome activation. Active caspase-1 causes not only cytotoxicity but also the secretion of the IL-1β and IL-18 inflammatory cytokines [2]. The latter repress IL-17 response, thereby impairing P.a. clearance *in vivo* [3,4].

The 8-hydroxyquinolines have shown inhibitory activity against T3SS of *Yersinia* and *Chlamydia* spp [5]. In a previous study (unpublished data), we have shown that the 8-hydroxyquinoline INP1855 decreased T3SS functionality *in vitro*.

OBJECTIVES

To study the effect of the T3SS inhibitor INP1855 :

- on the cytotoxicity of lab. strains and clinical isolates of P.a. towards epithelial and phagocytic cells (*in vitro*)
- on mice infected by T3SS+ or T3SS- strains (*in vivo*)

METHODS

Strains: CHA (clinical isolate expressing T3SS) and derivatives thereof (CHAΔSTY [no toxin production]; CHAΔExsA [deletion of T3SS regulon] and CHAΔpopBD [deletion of genes encoding translocation apparatus]); PA103 (cytotoxic strain expressing ExoU); PAO1 (reference strain); 12 clinical strains isolated from patients suffering from acute infections (respiratory tract; blood; wound; abdominal collection; urines; eye)

T3SS transcription: Real-time PCR of genes encoding toxins or translocon

Cells: human alveolar epithelial A549 cells; THP-1 monocytes

Cell viability: release of the cytosolic enzyme lactate dehydrogenase (LDH) in the culture medium after 7 h (A549 cells) or 5 h (THP-1 monocytes) of incubation *P.a.* strains (10 bact./cell).

In vivo assay: Mouse model of acute lung aggression induced by CHA (T3SS+) 5.10e6 CFU or CHAΔpopBD (T3SS-) 1.10e8 CFU with co-instillation of bacteria and INP1855 200 µM. After 16 h, mice were sacrificed and different parameters were evaluated (bacterial burden, lung lesion, bacterial dissemination). Cytokines measured by ELISA in bronchoalveolar lavage (BAL).

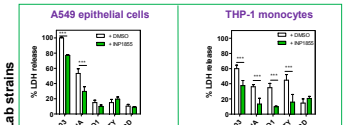
This poster will be made available for download after the meeting at : http://www.facm.ucl.ac.be/posters

IN VITRO RESULTS

Lab strains	mRNA expression		
	T3SS*	exoU*	exoS*
PA103	+	+	-
CHA	+	-	++
PAO1	+	-	+
CHA ΔSTY°	-	-	-
CHA ΔpopBD°	-	-	+

* expression of genes encoding for translocation apparatus (*popB, popD,pcrV*)
* expression of genes encoding ExoS or ExoU toxins
° no toxin produced but functional secretion apparatus
° toxins produced but not translocated in the host cells (no functional secretion apparatus)

Lab strains

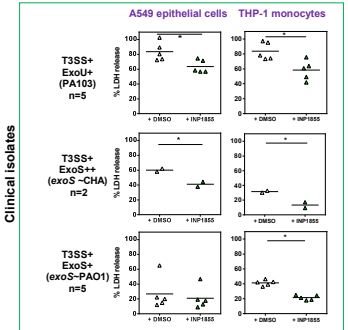


Toxicity is related to ExoU (PA103) or ExoS (CHA) toxin release in the cytosol.

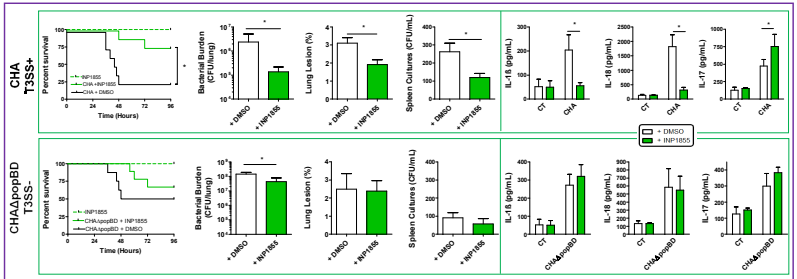
Toxicity relies either on ExoU or on the expression of translocation apparatus (CHA and CHAΔSTY) via inflammasome activation

INP1855 ↘ T3SS cytotoxicity (toxin- and/or inflammasome- dependent)

Clinical isolates

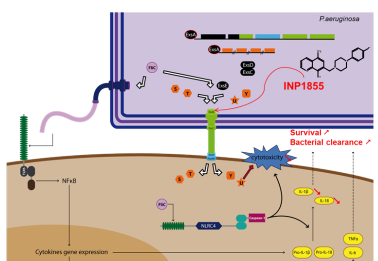


IN VIVO RESULTS



INP1855 ↗ mice survival and bacterial clearance for CHA T3SS+ strains

INP1855 ↘ IL-1β and IL-18 secretion and ↗ IL-17 secretion for CHA T3SS+ strains. No effect on CHAΔpopBD T3SS- strain



Effect of INP1855 as Type III secretion system inhibitor

CONCLUSION

- ↘ T3SS Functionality by INP1855
- ↘ Toxins translocation
- ↘ FliC or inner rod proteins translocation
- ↘ Inflammasome activation
  ↗ IL-17 secretion
- ↘ Cytotoxicity lab strains and clinical isolates (*in vitro*)
  ↘ Pulmonary lesions and dissemination (*in vivo*)