Salicylidene acylhydrazides and hydroxyquinolines decrease type three secretion system (T3SS)-dependent cytotoxicity induced by Pseudomonas aeruginos (P.a) clinical isolates

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INTRODUCTION AND OBJECTIVE

Expression of T3SS in P.a is associated with poor clinical outcome and high morbidity in acute infections. T3SS allows bacteria to inject toxins (e.g. ExoU or ExoS) into the host cell cytoplasm, causing cytotoxicity and preventing Pa internalization (1). T3SS can also deliver proteins like flagellin into the cytoplasm of phagocytic cells, inducing caspase-1 proteolysis via NLRCR4 inflammasome activation. Active caspase-1 causes not only cytotoxicity but also the secretion of IL-1β and IL-18, thereby impairing Pa clearance (2,3). Using THP-1 monocytes, we previously distinguished T3SS+ strains expressing ExoU (high cytotoxicity, causing cell death without inflammasome activation [T3SS+ExoU+]) from those expressing ExoU or no toxins (moderate cytotoxicity, decreased by caspase-1 inhibitor and thus related to inflammasome activation [T3SS+ExoU-]) (4). However, in epithelial cells (not expressing NLRC4 inflammasome), Pa cytotoxicity was only T3SS toxin-dependent.

Here, we compare the protective effects of two T3SS inhibitors (INP0341 [salicydene acylhydrazide (5)] and INP1855 [hydroxyquinoline (6)]) to caspase-1 inhibitor on inflammasome activation and cytotoxicity caused by Pa clinical isolates differing in their expression of T3SS and antimicrobial susceptibility.

MATERIALS & METHODS

Strains: CHA (clinical isolate expressing T3SS) and PA103 (clinical isolate expressing T3SS and ExoS). Clinical isolates of Pseudomonas aeruginosa are mainly resistant to the most commonly used antibiotics. MICs values and susceptibility patterns of the studied clinical isolates are shown in Table 1.

RESULTS

Cytotoxicity induced by Pa clinical isolates

Table: MICs values and susceptibility patterns of the studied clinical isolates of Pseudomonas aeruginosa

<table>
<thead>
<tr>
<th>Strains</th>
<th>MICs (µg/ml)</th>
<th>Resistance profile</th>
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<tbody>
<tr>
<td>CHA</td>
<td>2</td>
<td>Amikacin-resistant</td>
</tr>
<tr>
<td>PA103</td>
<td>4</td>
<td>Meropenem-resistant</td>
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<tr>
<td>Clinical isolates</td>
<td>0.5-16</td>
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Inflammasome activation induced by Pa clinical isolates

Conclusions

- **T3SS+ ExoU- isolates**: cytotoxicity, causing cell death without inflammasome activation
- **T3SS+ ExoU+ isolates**: moderate cytotoxicity, related to inflammasome activation, which induces IL-1β secretion.
- Both INP0341 and INP1855 protect eukaryotic cells from the toxic effects of Pa clinical isolates mediated by the T3SS toxins (ExoU and ExoS) or by inflammasome activation. INP1855 is more potent than INP0341.
- Protective effects are independent of the antibiotic resistance profile of the isolates.

- Inhibiting T3SS is thus a promising strategy deserving further evaluation in models of acute infections.

**References**