Pyocyanin-induced reduction of pro-inflammatory mediators in peritoneal macrophages

Sales-Neto, J.M1, Lima, É.A1, Cavalcante-Silva, L.H.A1, Vasconcelos, U1, Rodrigues-Maia, S1

Abstract

Bacterial pathogen Pseudomonas aeruginosa (PA), which is considered a major virulence factor, PA infection induces reactive oxygen species (ROS) increase, causing tissue inflammation and neutrophil apoptosis. Inflammation is a beneficial response to external challenge or cellular injury that involves macrophage activation, leads to the release of several inflammatory mediators and restoration of tissue structure and function. Little is known about the mechanisms triggered by PCN on activated macrophages. Thus, the aim of this work was to evaluate, in vitro, the role of PCN on nitric oxide (NO) production, interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) secretion by macrophages. Peritoneal exudate macrophages were obtained 4 days after i.p. injection of 2 mL of thioglycolate. PCN cytotoxicity to LPS activated macrophages was tested by MTT after incubation of macrophages with 1 to 100 μM PCN. Additionally, cytokines (IL-1β and TNF-α) concentrations were assayed using ELISA kits and NO production by nitrite accumulation. PCN high concentrations treatment (50 and 100 μM) was cytotoxic. On the other hand, up to 10 μM, PCN does not affect cell viability. PCN (5 and 10 μM) attenuates NO production, 26.13 % and 51.02 %, respectively. Furthermore, PCN (1, 5, and 10 μM) attenuates TNF-α and IL-1β levels, 47.63 e 37.53 %, respectively. Together these data suggest an anti-inflammatory role of PCN, which might be related to host immune response suppression mechanism and PA infection persistence.

Keywords: Pseudomonas aeruginosa. Pyocyanin. Nitric oxide. Pro-inflammatory cytokines. Macrophages.

Introduction

The major clinical problem for cystic fibrosis patients is a progressive loss of lung function caused by chronic lung infection with mucoid Pseudomonas aeruginosa (PA). PA produces several pigments while growing, such as fluorescein (yellow-green), pyomelanin (brown-red), and pyocyanin PCN (blue-green), which is the major virulence factor (Gibson; Burns; Ramsey, 2003). The repeated cycles of pro- and anti-inflammatory responses triggered by P. aeruginosa-associated secreted exoproducts, progressively compile the damage on CF lungs. PCN and other virulence factors are associated with infectivity, contributing to establishing and reestablishing of PA infections (Caldas; Boisramé, 2015). PCN also enhances superoxide production, which can affect cellular immune functions of the host causing neutrophil apoptosis. A direct evidence for the importance of PCN on the PA-infected airway was suggested by Lau and co-workers (Lau et al, 2004). Macrophages and inflammatory mediators, such as NO, IL-1β, and TNF-α, are important for the inflammatory response after PA infection (Friedlander et al., 1994). In this study, we examined whether PCN could interfere on peritoneal macrophage-derived inflammatory mediators and NO production.

Material and Methods

Female Swiss albino mice (2 months old) were obtained from Thomas George animal house of CBio- tec, UFPB. All procedures adopted were approved by Ethics Committee (CEUA: 99/2015). Peritoneal exudate was elicited with an i.p. injection of 2 mL of thioglycolate. PCN cytotoxicity to LPS activated macrophages was tested by MTT after incubation of macrophages with 1 to 100 μM PCN. Additionally, cytokines (IL-1β and TNF-α) concentrations were assayed using ELISA kits and NO production by nitrite accumulation. PCN high concentrations treatment (50 and 100 μM) was cytotoxic. On the other hand, up to 10 μM, PCN does not affect cell viability. PCN (5 and 10 μM) attenuates NO production, 26.13 % and 51.02 %, respectively. Furthermore, PCN (1, 5, and 10 μM) attenuates TNF-α and IL-1β levels, 47.63 e 37.53 %, respectively. Together these data suggest an anti-inflammatory role of PCN, which might be related to host immune response suppression mechanism and PA infection persistence.

Results are expressed as mean ± SEM and significance of difference was determined by one-way ANOVA and post hoc Tukey multiple comparisons test.
Results
PCN (50 and 100 μM) is capable to induce cell death (90 and 95% respectively), while 1, 5, and 10 μM PCN does not affect cell viability (data not shown). Additivehal, 5 (26.13 %, p < 0.05) and 10 μM (51.02 %, p < 0.0001) PCN reduced NO generation (Figure 1A). Furthermore, treatment with 1, 5, and 10 μM PCN reduced IL-1β (37.53 % at 5 μM) and TNF-α (47.63 % at 5 μM) levels on LPS activated macrophages (Figure 1B and Figure 1C).

Discussion and Conclusion
During inflammation, a complex process of intracellular signal transduction and transcription events, driven by multiple proinflammatory mediators and cytokines, is activated (Dmitrieva et al., 2016). Macrophages play an important role in controlling this process, secreting NO and pro-inflammatory cytokines, such as IL-1 and TNF-α, leading to leukocytes infiltration and immune response against the pathogen (Cassado; Lima; Bortoluci, 2015). The results of these experiments indicate that PCN reduces NO and pro-inflammatory cytokines levels, IL-1β and TNF-α. Besides that, PCN was also able to inhibit NO production on alveolar macrophages (Shellito; Nelson; Sorensen, 1992) and prostacyclin production (Kamath et al., 1995). This effect may be a means of evading host defenses against infection, contributing to the establishment of bacterial infection. However, further studies are necessary to elucidate the mechanisms involved.

References
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