Pyocyanin pigment from *Pseudomonas aeruginosa* modulates innate immune defenses in macrophages

Yasmine K. Mahgoub#, Rida Arif#, Susu M. Zughaier*

College of Medicine, QU Health, Qatar University, Doha, Qatar, 2731

 # Authors contributed equally; * corresponding author (szughaier@qu.edu.qa)

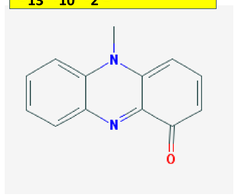
Introduction

Background: Pseudomonas aeruginosa is a well-known opportunistic pathogen. The gram-negative bacillus, commonly associated with hospital-acquired infections, utilizes the host's impaired immune responses to establish infection. Of its many virulence factors, pyocyanin is essential for P. aeruginosa to establish its full infectivity. Macrophages act as sentinels of the innate immune system, as well as play other roles in homeostasis, tissue remodelling, and bridging between the innate and adaptive immune systems.

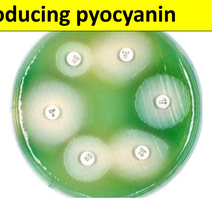
Purpose: This study aimed to investigate the effects of pyocyanin on macrophage innate immune defences by assessing the function of macrophages treated with pyocyanin and TLR ligands. Phagocytosis of opsonized zymosan, LPS-induced nitric oxide release and cytokine release were used as measures of functional responses.

Pyocyanin (PCN) is a major virulence factor

Pyocyanin (PCN) $C_{13}H_{10}N_2O$



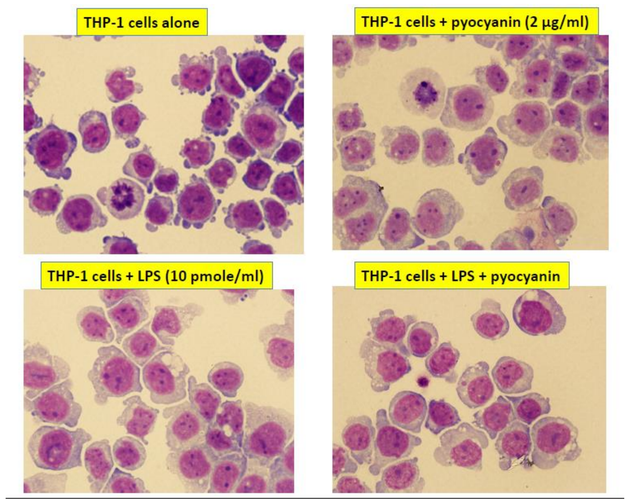
Pseudomonas aeruginosa producing pyocyanin



- PCN is essential for *P. aeruginosa* virulence
- PCN is important for biofilm development
- PCN is controlled by quorum sensing process
- PCN is cytotoxic redox-active small molecule

PCN inhibits catalase; oxidizes glutathione, reduces NADPH thus depletes substrate for NADPH oxidases

PCN is toxic to mammalian cells at high doses, No aberrant cytotoxicty with low dose of PCN



Results

Pyocyanin inhibits, in a dose-dependent manner, ROS release from macrophages

Upon phagocytosis of invading pathogens, respiratory burst is triggered as a defense mechanism that leads to release of reactive oxygen species (ROS) which is important for the oxidative killing of invading pathogens.

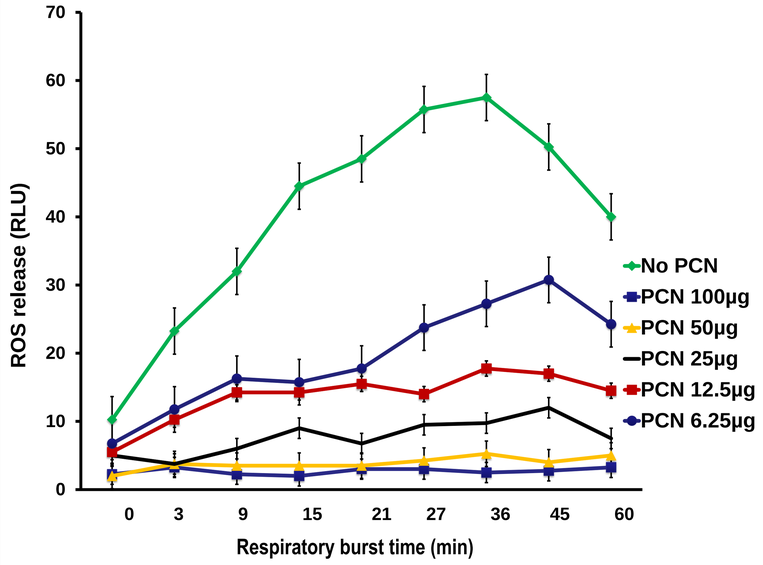


Figure 1: THP-1 cells primed with *P. aeruginosa*-derived LPS (10 pmole/ml) overnight. Respiratory burst was triggered with opsonized zymosan and ROS release was detected using the chemiluminescent probe lucigenin. Pyocyanin doses were added at the start of respiratory burst and ROS release was monitored for 1 hour. The data suggest that pyocyanin inhibits the respiratory burst possibly by reducing NADPH and depleting substrate for NADPH oxidases which reduces ROS release and spare the oxidative killing of invading *P. aeruginosa*.

Pyocyanin inhibits LPS-induced nitric oxide release from macrophages

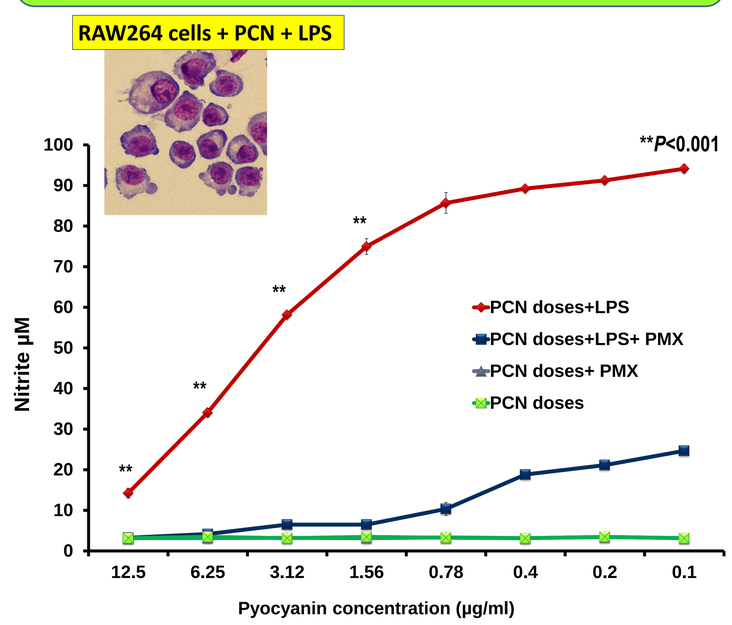
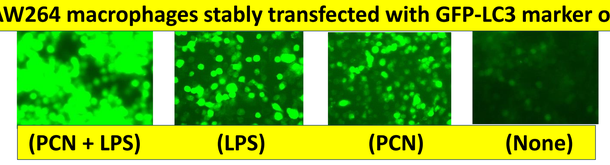


Figure 2: Murine RAW264 macrophages were stimulated with with *P. aeruginosa*-derived LPS (10 pmole/ml) overnight in presence of pyocyanin (PCN) doses. Polymyxin (PMX) , a known inhibitor of LPS activity, was used at 2 µg/ml as a positive control. Nitric oxide release was determined as nitrite accumulation and measured by the Griess method. The data suggest that pyocyanin modulates macrophage innate immune responses.

Pyocyanin amplifies autophagy formation

RAW264 macrophages stably transfected with GFP-LC3 marker of autophagy



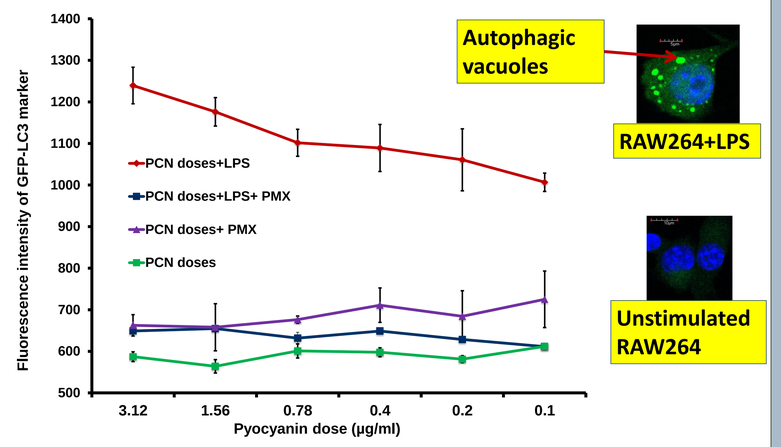


Figure 3: Murine RAW264 macrophages stably transfected with the autophagy marker GFP-LC3 were stimulated with *P. aeruginosa*-derived LPS (10 pmole/ml) with or without pyocyanin doses and incubated overnight. Autophagy formation was visualized as LC3 aggregates or puncta using fluorescence microscopy imaging. GFP-LC3 fluorescence intensity was quantified using fluorescence plate reader with excitation at 485 nm and emission at 528 nm. The data suggest that pyocyanin amplify autophagy formation which may be due to PCN's ability to inhibit cellular respiration in mitochondria, since damaged mitochondria are recycled via autophagy.

PCN reduced TNFα release from human monocytes

TNFα is an essential mediator in the inflammatory pathway initiated by the immune system. Inducers (e.g. LPS) signal the de novo synthesis of inflammatory cytokines such as TNF α from activated macrophages which then participates in many different aspects of inflammation.

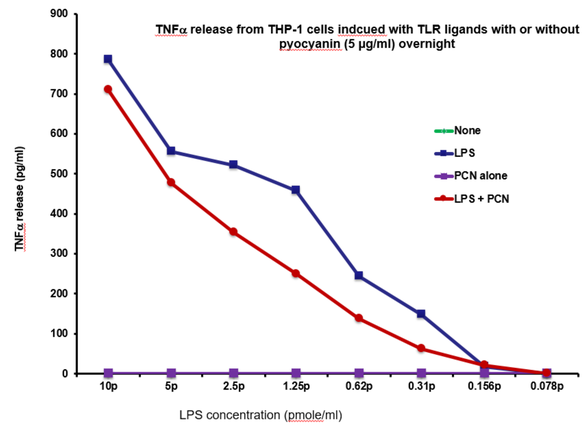


Figure 4 PCN inhibits TNFα release from human macrophage-like monocytic cells THP-1.

Conclusion

Pyocyanin inhibits macrophage functional defense responses to facilitate *Pseudomonas aeruginosa* infection.