The β-lactamase Inhibitor Boronic Acid SM23 Inhibits *Pseudomonas aeruginosa* Biofilm Formation and Virulence Factor Production

Samuele PEPPOLONI[1], Eva PERICOLINI[1], Bruna COLOMBARI[1], Diego PINETTI[2], Claudio CERMELLI[1], Francesco FINI[3], Fabio PRATI[3], Emilia CASELLI[3] and Elisabetta BLASI[1]*

[1]Department of Surgical, Medical, Dental and Morphological Sciences with interest in Transplant, Oncological and Regenerative Medicine, University of Modena and Reggio Emilia, Modena, Italy;
[2]Centro Interdipartimentale Grandi Strumenti (CIGS), University of Modena and Reggio Emilia, Modena, Italy;
[3]Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy.

Introduction: *Pseudomonas aeruginosa* is a Gram-negative nosocomial pathogen, often responsible of severe device-related infections, given its great ability to produce biofilm. *P. aeruginosa* finely regulates the expression of different virulence factors, including biofilm production, by Quorum Sensing (QS), an intercellular communication mechanism used by many bacteria. Biofilm formation enhances bacterial resistance to antimicrobial agents due to a decreased penetration of antibiotics and a reduced rate of growth of embedded bacteria. Thus, novel agents capable of selective inhibiting biofilm formation may represent a promising strategy to overcome the well-known and widespread drug-resistance of *P. aeruginosa*.
Material and Methods: by using the bioluminescent *P. aeruginosa* strain P1242, we investigated the effects of SM23, a boronic acid derivative specifically designed as beta-lactamase inhibitor, on biofilm formation and virulence factor production by *P. aeruginosa*.
Results: we found that SM23: a) inhibited both biofilm formation and production of the virulence factors, pyoverdine, elastase and pyocyanin, without affecting bacterial growth; b) decreased the levels of QS-related autoinducers molecules, namely *3-oxo-C_{12}-HSL* and *C_4-HSL*, by reducing *lasR*/*lasI* system gene expression in the biofilm; c) failed to bind to bacterial cells that had been preincubated with *P. aeruginosa*-conditioned medium; d) reduced significantly *P. aeruginosa* biofilm and pyoverdine production on endotracheal tubes, an *in vitro* condition closely mimicking clinical settings.
Discussion and Conclusions: taken together, our results indicate that, besides inhibiting beta-lactamase, the boronic acid SM23, can also act as potent inhibitor of *P. aeruginosa* virulence, by profoundly affecting biofilm and QS-related signals. These findings highlight potential application of this compound in the prevention and treatment of biofilm-associated *P. aeruginosa* infections.