Iron chelation directed against biofilms as an adjunct to conventional antibiotics

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TO THE EDITOR: We read with interest the study by Moreau-Marquis et al., demonstrating the bactericidal effect of two iron-chelating compounds directed against Pseudomonas aeruginosa when combined with the aminoglycoside antibiotic tobramycin, following their recent demonstration of abnormal cystic fibrosis epithelial cell iron hardling (1, 2). In similar experiments to those of Moreau-Marquis and colleagues, we assessed the effects of the synthetic iron chelator 2,2-dipyridyl (2DP) on minimal inhibitory concentrations (MICs) of ceftazidime and meropenem. We used a broth microdilution technique in medium containing low (≤ 1 μM) or high (10 μM) amounts of iron (FeCl3) and examined growth and biofilm formation under aerobic and anaerobic conditions. The inclusion of anaerobic experiments is critical, as these conditions are likely encountered by P. aeruginosa in the cystic fibrosis (CF) lung environment, and anaerobic growth within biofilms is thought to be a critical factor in persistence (4, 5). Our data demonstrate that the iron requirement for growth and biofilm formation is much higher when bacteria are grown anaerobically compared with aerobically (O’May, unpublished observations). Under aerobic conditions, iron had little effect on MICs, but when P. aeruginosa strains were grown anaerobically in the presence of supplemental iron, the MICs were significantly increased to the point where the strains were considered to be resistant. The antimicrobial resistance of strains to ceftazidime and meropenem was significantly decreased (up to 4-fold) under both aerobic and anaerobic growth conditions by the addition of 2DP at levels that did not inhibit P. aeruginosa growth. Interestingly, under anaerobic conditions, the concentration of 2DP required to achieve the same reduction in MIC was much lower than under aerobic conditions.

In experiments using a flow cell biofilm model in which bacteria were grown on glass surfaces and stained for viability (live P. aeruginosa stains green and dead bacteria stain red; BacLight), we have demonstrated that a combination of 2DP and tobramycin results in areas of denuded biofilm in close proximity to dead bacteria (see Fig. 1). In contrast, tobramycin on its own had no effect. The 2DP and tobramycin combination was superior to 2DP alone. We have previously reported, using this model, that 2DP can reduce P. aeruginosa biofilm mass and height, but not bacterial viability or biofilm surface area coverage (chelator added to mature biofilms formed over 2–3 days) (O’May, unpublished observations). Our flow cell experiments imply that iron-chelation disrupts biofilm structure, which then allows antibiotic penetration and bacterial killing. In the flow cell method, biofilms are mature and in their most resistant form when chelator-antibiotic interventions are introduced and complement the results from the model of Moreau-Marquis and colleagues where biofilms were assessed at an early stage of development (≤ 6 h).

Collectively, the data of our studies (3) and those of Moreau-Marquis et al. (1) show that combination therapy with iron-chelators and conventional antibiotics may be particularly effective in the CF lung, especially in view of the increased amounts of iron available to support P. aeruginosa replication and biofilm formation in this disease. Our data also suggest that iron chelation may enhance the efficacy of antibiotics such as tobramycin under low oxygen tensions, which normally impair the bactericidal effects of the aminoglycosides.

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REFERENCES


