Investigating the effect of tobramycin dry powder inhaler on the eradication of Pseudomonas aeruginosa biofilms.

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Introduction

- Biofilms are sessile communities of microorganisms embedded within a self-generated extracellular polymeric matrix. Such biofilms are found for instance in cystic fibrosis patients, with pulmonary infections caused by the Gram-negative bacterium Pseudomonas aeruginosa being particularly common.
- Lung infections in CF patients are commonly managed using antibiotic dry powder inhalers, one of which is the aminoglycoside tobramycin. The activity of tobramycin has been well characterized in vitro, but current models that have been used are not very representative for lung infections, and better models would provide significant advantages.
- Our aim is to develop a biofilm model that enables us to test dry powder inhalers. Such as investigating the effect of particle size deposited from a dry powder inhaler antibiotic, tobramycin, on eradication of biofilms.

Method

- A Next Generation Impactor (NGI) was used to fractionate tobramycin generated from TOBI Podahler® inhalation powder into size fractions. NGI consists of several nozzles with progressively reducing jet diameters, with differently-sized particles collected at different stages. (Figure 1).
- Tobramycin capsules were manually loaded and fired into the NGI at a flow rate of 30 or 60 L/min. These particles were collected on glass fibre filters that were mounted on the NGI. The mass of the collected tobramycin was quantified using HPLC-MS.
- Different particle sizes of tobramycin powder were applied on P. aeruginosa biofilms for 24h. The biofilms were previously grown and inoculated into polycarbonate membranes for 48h using a colony biofilm assay. Then Colony Forming Unit (CFU) was calculated.

Result 1. Scanning electron microscopy (SEM) of tobramycin particles

- SEM images were taken to visualise tobramycin particles (Figure 2) and to calculate the size distribution using ImageJ (Figure 3), (Table. I).

Result 2. Tobramycin particle size distribution

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Result 3. Tobramycin masses at different cut-off diameters

- Similar masses (~0.990mg) with different cut-off diameters were collected from firing 4.4mg and 2.5mg tobramycin capsules at a flow rate of 30 or 60 L/min respectively. (Figure 4).

Result 4. Anti-biofilm activity of different tobramycin particle sizes

- In all tested clinical isolates, but not the laboratory strain PAO1, we found that there is a trend that smaller-size tobramycin micro-particles (< 1.4 μm) being more effective in eradicating biofilm as compared to the larger tobramycin micro-particles (< 5.5 μm) (Figure 5). This indicates that the particle size has an effect on the anti-biofilm activity of tobramycin but more investigations are needed to clearly verify this effect.

Conclusion and future work

- Preliminary data suggest that there is a trend that smaller-sized tobramycin particles are more effective in eradicating of P. aeruginosa biofilms as compared to larger particles but more tests are required to prove that.
- Future work will include testing the influence of a wider range of tobramycin particles sizes against P. aeruginosa biofilms to build up a more accurate picture about the relationship between particle size and its anti-biofilm activity. This will not only tested for tobramycin, but also other dry powder antibiotics.

References


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