EFFECT OF (E,Z)-4,5,9-TRITHIADODECA-1,6,11-TRIENE 9-OXIDE IN *IN VITRO* AND *IN VIVO* MODELS OF *PSEUDOMONAS AERUGINOSA* INFECTION

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BACKGROUND

Pseudomonas aeruginosa is an opportunistic pathogen that causes a variety of infections in humans and is the leading cause of lung infections in people with cystic fibrosis. Once established, these infections can be lifelong and despite intensive antibiotic treatment remain established in the respiratory tract. Over time, the *P.aeruginosa* populations display impressive diversity which both allows adaptation to the respiratory niche and selection of resistant isolates. In this study we assessed the antimicrobial properties of (E,Z)-4,5,9-trithiadodeca-1,6,11-triene 9-oxide (NXAS401) a small molecule quorum sensing inhibitor.

METHODS

Anti-biofilm properties of NXAS401 (Fig. 1) were assessed against a 7-day biofilm formation model and a pre-formed biofilm of *P.aeruginosa* (LESB65) in artificial sputum media (ASM). Effects on CFUs and pyocyanin production were quantified following single treatment with NXAS401 or Tobramycin, compared to untreated controls. Antimicrobial enhancement was assessed by combined treatment of NXAS401 and Tobramycin.[1]

Fig. 1: Chemical structure of (E,Z)-4,5,9-trithiadodeca-1,6,11-triene 9-oxide, Z isomer.

Expression of genes involved in QS (*lasA, lasR, rhlR*), exopolysaccharide production (*phzF*), biofilm formation (*algD, flgD, pslD*) and CFTR inhibition (*cif*), was quantified at 1, 3 & 7 days post-treatment using RT-qPCR.[2]

The effect of NXAS401 on bacterial clearance was studied in a chronic *P.aeruginosa* (LESB65) *in vivo* model. Male BALB/c mice were infected with 10^6 CFU/ml by intranasal instillation. Treatment occured on days 2, 4 & 6 post infection, with PBS, NXAS401, Tobramycin or Tobramycin + NXAS401. CFUs were counted on days 3, 5, & 7.[3]

ASM

7-day biofilm model

Tobramycin + NXAS401 reduced the number of CFU on day 1 and 2 and reduced pyocyanin production from day 2, compared to treatment with Tobramycin alone (Fig 2).

Pre-formed biofilm model

Combined treatment enhanced the effect of Tobramycin on day 2. Pyocyanin production was reduced on day 2 and 3, though did not reach statistical significance (Fig 3).

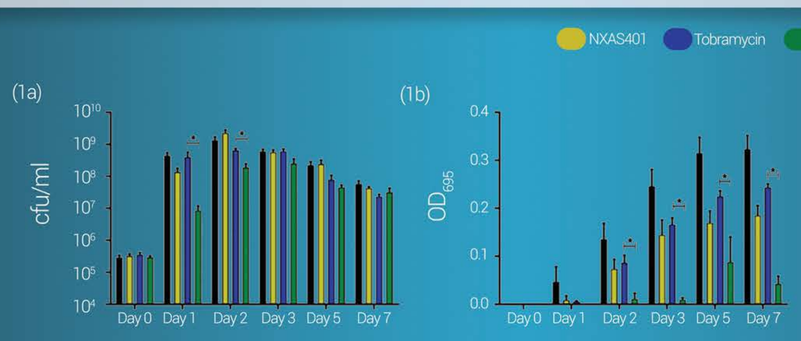

Fig. 2: 7-day biofilm model.
(1a) cfu/ml of *P.aeruginosa* and (1b) optical density measure of pyocyanin production, following single or combined treatment (* $p < 0.05$).

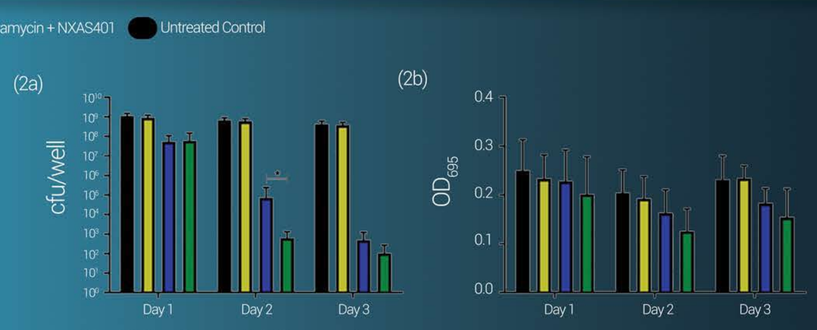

Fig. 3: Pre-formed biofilm model.
(2a) cfu/ml and (2b) optical density measure of pyocyanin production, following 3 consecutive days of single or combined treatment (* $p < 0.05$).

GENE EXPRESSION

NXAS401 treatment reduced expression of all studied genes 3 days following treatment. Tobramycin + NXAS401 led to a long-term decrease in expression of *lasA, rhlR, phzF* and *cif*, evident at day 7, compared to Tobramycin alone.

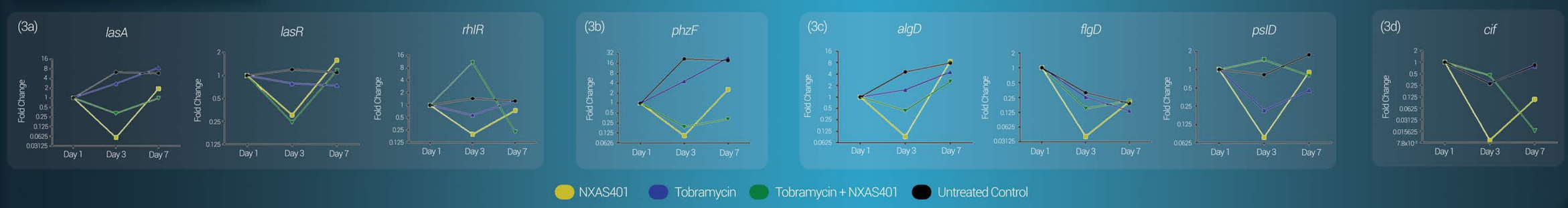

Fig. 4: Fold change in gene expression of P. aeruginosa at day 3 & 7 following treatment, normalised to day 1 for genes related to (3a) QS; (3b) pyocyanin production; (3c) biofilm formation; (3d) CFTR inhibition.

BACTERIAL CLEARANCE *IN VIVO*

Tobramycin + NXAS401 increased the rate of bacterial clearance from the nasopharynx of infected mice, with a trend towards fewer cfu/nasopharynx on individual days 3 and 5.

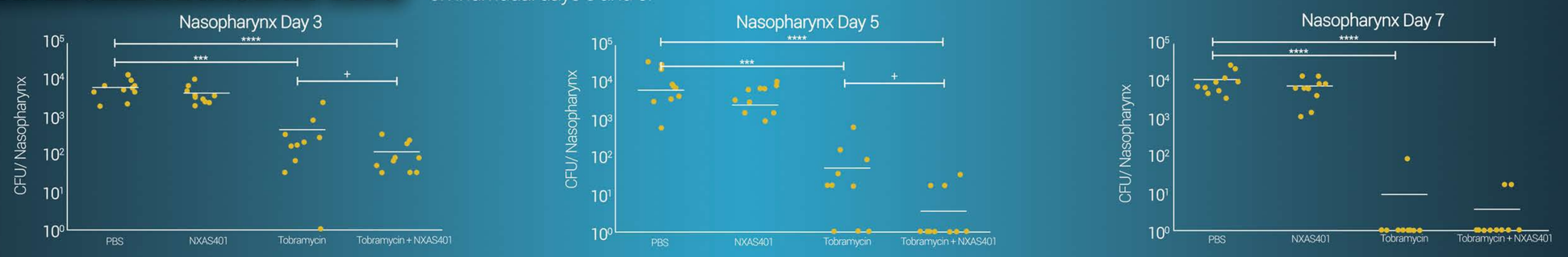

Fig. 5: cfu/nasopharynx of *P.aeruginosa* infected BALB/c mice at days 3, 5 and 7.

(+ $p < 0.1$, *** $p < 0.001$, **** $p < 0.0001$.)

CONCLUSIONS

- NXAS401 enhances the anti-biofilm properties of Tobramycin *in vitro* and significantly inhibits the production of pyocyanin when administered in combination with Tobramycin.

- NXAS401 induces down-regulation of genes involved in the Quorum Sensing system (*lasA, lasR, rhlR*), biofilm formation (*algD, flgD, pslD*) and CFTR inhibition (*cif*).

- Treatment with the Tobramycin + NXAS401 combination *in vivo* leads to faster clearance from the nasopharynx of BALB/c mice.

- NXAS401 has been awarded Orphan Drug Designation by the FDA for the treatment of *P.aeruginosa* lung infections in people with cystic fibrosis.

REFERENCES: [1] *J. Vis. Exp. (64), e3857, doi:10.3791/3857 (2012).* [2] *Antimicrob Agents Chemother, 56: 2314-25. (2012).* [3] *Nature Commun. 5:4780 (2014).*