Airway microbiome studies challenge simplistic models of inhaled tobramycin benefit

Geraint B Rogers

In 1999, Ramsey and colleagues published the results of a clinical trial of aerosolised tobramycin in cystic fibrosis (CF) to target chronic *Pseudomonas aeruginosa* infection.[1] In keeping with their earlier demonstration of efficacy,[2] they reported treatment to be associated with a reduction in *P. aeruginosa* sputum density and substantial clinical benefit (improved lung function and reduced risk of hospitalisation). Consistent findings were reported in subsequent studies,[3 4] leading tobramycin inhaled powder/solution (TIP/S) maintenance therapy to go on to become the most common antibiotic used to treat people with cystic fibrosis (PWCF). While none of these studies related clinical outcome to microbiological impact directly, it is widely assumed that depletion of *P. aeruginosa* underpins TIP/S benefit.

This presumptive mechanism of TIP/S, and its limitation to those with chronic *P. aeruginosa* infection, has been challenged by two recent studies. In this issue, Heirali and colleagues report an investigation of the microbial predictors of response to TIP/S in adults with CF.[5] 16S rRNA gene amplicon sequencing was applied to banked sputum samples from 41 patients with chronic *P. aeruginosa* infection who had received TIP/S for at least 1 year and from whom sputum samples were available before and after the initiation of therapy. Somewhat unexpectedly, their study determined response to therapy (defined by the absence of a net decrease in forced expiratory volume in one second (FEV_1)) to be associated with higher relative abundance of staphylococci in sputum at baseline. In contrast, non-responders trended towards having a higher relative abundance *P. aeruginosa* (indeed, lower *Pseudomonas* abundance was associated with response in all but the a priori response definition). Analysis of samples collected before and after TIP/S initiation showed airway microbiology to not change substantially. That staphylococcal levels predict response is perhaps not surprising, given that members of this genus are rare among Gram-positive respiratory pathogens in being sensitive to tobramycin. However, the failure to observe a reduction in the levels of *Staphylococcus* with treatment suggests a mechanism beyond a simple antimicrobial effect.

In a separate study, Nelson and colleagues set out to determine the microbiological impact of TIP/S in PWCF over the course of a standard month-long course of maintenance therapy.[6] Combined application of culture, amplicon sequencing, and metagenomic analysis (modified to assess intact bacterial cells) to weekly sputum samples collected from 30 individuals revealed that principal microbiological changes (occurring between baseline and week one of therapy) did not relate to *P. aeruginosa* or *Staphylococcus aureus*, but largely involved the depletion of non-dominant, facultative and obligate anaerobes. In fact, based on molecular analysis, no classic CF pathogens showed a significant change in abundance in response to treatment. Furthermore, no differences in baseline microbiota composition, or change in sputum microbiology after 1 week of therapy, were identified between non-responders and responders (defined as the upper tertile of per cent predicted FEV_1 change), and no association was found between microbiological changes and symptomatic responses.

P. aeruginosa levels not predicting response to TIP/S, and not declining as a result of treatment, is inconsistent with our current understanding of the mechanism of clinical benefit. It could be concluded from the findings of Heirali *et al* that the association between pretreatment staphylococcal abundance and therapeutic response suggests TIP/S provides benefit through a direct antibiotic impact on airway pathogens other than *P. aeruginosa*. However, it is notable that no association between treatment response and a reduction in *Staphylococcus* was reported in either study. Perhaps these findings should not be so unexpected, given that there is surprisingly little, if any, evidence to directly link pseudomonal depletion and clinical benefit from TIP/S, despite this presumed mechanism underpinning its use. Indeed, it is notable that phenotypical tobramycin resistance in *P. aeruginosa* does not appear to predict clinical benefit.

The failure to observe a reduction in *P. aeruginosa* levels in either study could reflect the many adaptations that this pathogen undergoes in the CF airways that reduce its susceptibility to tobramycin.[7] The effects of such adaptations would be consistent with the marked reduction in *P. aeruginosa* levels achieved with TIP/S during early stages of infection.[8]

So how might the clinical benefit reported in these two studies be best explained? One possibility is that TIP/S results in a reduced expression of pathogenicity traits by *P. aeruginosa* or *S. aureus*, without substantial cell death. By diverting cellular resources from the production of proinflammatory factors, the upregulation of resistance mechanisms, such as efflux pumps, could contribute to such an effect. Disparities between the results of culture-based and molecular analysis reported by Nelson *et al* are also consistent with airway pathogens adopting viable but unculturable states, an adaptive response to antibiotic stress, further reducing antibiotic susceptibility and proinflammatory potential.

Changes in pathogen behaviour might also arise through to the impact of TIP/S on non-target bacteria. Nelson *et al* reported treatment to result in the depletion of taxa normally associated with the oral cavity and oropharynx (although it is unclear whether this occurs in the upper or lower airways). However, it is notable that contributors to the study by Heirali *et al* have previously demonstrated that the presence of avirulent oropharyngeal microbes modulates the expression of pseudomonal virulence in *P. aeruginosa* coinfection models.[9 10]

While such schemas are consistent with the absence of an observed reduction in pathogen load with TIP/S, they are not consistent with the failure of pretreatment *P. aeruginosa* levels to predict treatment response. An alternative explanation for both the relative predictive capacity of *Staphylococcus* and *Pseudomonas* and the absence of a correlation between pathogen depletion and clinical benefit is that these pathogens are associated with different levels of disease severity, and thereby the likelihood of an appreciable response to TIP/S. Tobramycin almost certainly reduces lung function decline by disrupting an ongoing cycle of airway inflammation and damage[2] (whether via bacterial depletion or downregulation of pathogenicity). Chronic *P. aeruginosa* infection is typically associated with later

Correspondence to Dr Geraint B Rogers, Microbiome Research, South Australian Health and Medical Research Institute, Adelaide, South Australia, Australia; geraint.rogers@sahmri.com

stage disease and more severe inflammation. The difference in the predictive values of *P. aeruginosa* and *S. aureus* levels might therefore relate to the ability of TIP/S to reduce inflammation and airway obstruction to a point where it is reflected in a slowing of lung function decline, with *S. aureus* indicating lower baseline levels. In such a scenario, *P. aeruginosa* baseline levels might predict clinical outcomes over the longer term.

The complexity of chronic CF lung infections and the considerable variation in disease characteristics between individuals frustrate efforts to understand mechanisms of antibiotic benefit. Models in which single interventions result in a uniform response, via a single mechanism, are typically overly simplistic. Further studies to better understand how TIP/S provides clinical benefit to PWCF with chronic *P. aeruginosa* infections are now clearly important. However, these two studies suggest the potential application of TIP/S beyond this pathogen and, perhaps extending to other non-CF clinical contexts, should be considered.

Contributors GBR was the sole contributor.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Commissioned; externally peer reviewed.

To cite Rogers GB. *Thorax* 2020;**75**:1031–1032.

Accepted 9 October 2020
Published Online First 2 November 2020

Linked

► http://dx.doi.org/10.1136/thoraxjnl-2019-214191
► http://dx.doi.org/10.1136/thoraxjnl-2019-214187

Thorax 2020;**75**:1031–1032.
doi:10.1136/thoraxjnl-2020-216005

REFERENCES

1 Ramsey BW, Pepe MS, Quan JM, *et al*. Intermittent administration of inhaled tobramycin in patients with cystic fibrosis. cystic fibrosis inhaled tobramycin Study Group. *N Engl J Med* 1999;340:23–30.
2 Ramsey BW, Dorkin HL, Eisenberg JD, *et al*. Efficacy of aerosolized tobramycin in patients with cystic fibrosis. *N Engl J Med* 1993;328:1740–6.
3 Konstan MW, Flume PA, Kappler M, *et al*. Safety, efficacy and convenience of tobramycin inhalation powder in cystic fibrosis patients: the EAGER trial. *J Cyst Fibros* 2011;10:54–61.
4 Konstan MW, Geller DE, Minić P, *et al*. Tobramycin inhalation powder for *P. aeruginosa* infection in cystic fibrosis: the EVOLVE trial. *Pediatr Pulmonol* 2011;46:230–8.
5 Heirali A, Thornton C, Acosta N, *et al*. Sputum microbiota in adults with CF associates with response to inhaled tobramycin. *Thorax* 2020;75:1058–64.
6 Nelson MT, Wolter DJ, Eng A, *et al*. Maintenance tobramycin primarily affects untargeted bacteria in the CF sputum microbiome. *Thorax* 2020;75:780–90.
7 Müller L, Murgia X, Siebenbürger L, *et al*. Human airway mucus alters susceptibility of Pseudomonas aeruginosa biofilms to tobramycin, but not colistin. *J Antimicrob Chemother* 2018;73:2762–9.
8 Gibson RL, Emerson J, McNamara S, *et al*. Significant microbiological effect of inhaled tobramycin in young children with cystic fibrosis. *Am J Respir Crit Care Med* 2003;167:841–9.
9 Duan K, Dammel C, Stein J, *et al*. Modulation of *Pseudomonas aeruginosa* gene expression by host microflora through interspecies communication. *Mol Microbiol* 2003;50:1477–91.
10 Sibley CD, Duan K, Fischer C, *et al*. Discerning the complexity of community interactions using a *Drosophila* model of polymicrobial infections. *PLoS Pathog* 2008;4:e1000184.