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Original Article

ORIGINAL ARTICLE

The renoprotective effect of concomitant fosfomycin in the treatment of pulmonary exacerbations in cystic fibrosis

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ABSTRACT

Background. Fosfomycin, effective in Cystic Fibrosis (CF), competes with aminoglycosides at renal binding sites and may therefore afford a renoprotective effect when used in combination therapy. We explored this by using markers of acute renal tubular damage [N-acetyl-β-D-glucose-aminidase (NAG), alanine amino-peptidase (AAP) and β_2-microglobulin].

Methods. Using a prospective randomized crossover trial design, at an acute pulmonary exacerbation, 18 adult CF patients received either 14 days of intravenous (IV) tobramycin or IV tobramycin and IV fosfomycin, both in combination with a second IV antibiotic (colomycin).

Results. Urinary NAG ($P = 0.003$) and AAP ($P = 0.03$) following treatment with concomitant fosfomycin were lower than those after treatment with tobramycin and colomycin alone. Fosfomycin attenuated the total 24-h urinary protein leak ($P = 0.0001$). The 14-day improvements in all surrogate markers of exacerbation resolution (FEV_1% predicted, FVC, white cell count and C-reactive protein) were similar for both treatment regimens.

Conclusions. The addition of fosfomycin reduces acute renal injury caused by IV aminoglycoside therapy in CF pulmonary exacerbations.

Keywords: acute tubular necrosis, cystic fibrosis, fosfomycin, pulmonary exacerbations, renoprotection

INTRODUCTION

Effective antibiotic therapy is critical in CF, where pulmonary exacerbations typically caused by bacteria such as *Pseudomonas aeruginosa* (Psa) are associated with clinical decline and increased morbidity.

Multiresistant isolates of Psa are an increasing problem. The most effective treatment regimen in CF pulmonary Psa exacerbations is still uncertain. It is recommended that respiratory exacerbations are treated with two intravenous (IV) antibiotics to minimize the risk of antibiotic resistance developing [1, 2].

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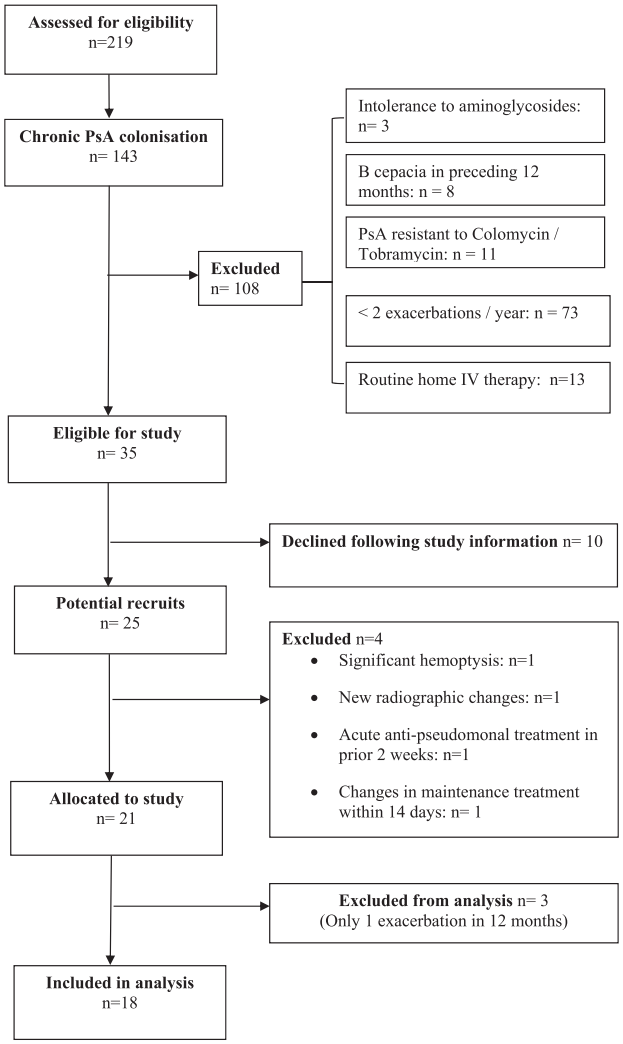

FIGURE 1: Flow diagram of study selection.

Combined therapy may have an additive or synergistic effect. The initial choice of antibiotic still depends on *in vitro* bacterial sensitivity patterns and often includes an aminoglycoside. Although aminoglycosides are powerful anti-pseudomonal agents and their use in CF is widespread and unavoidable, they are resorbed by the proximal renal tubule and have the potential for nephrotoxicity. Indeed, acute renal injury is common and we have previously shown a link between repeated IV aminoglycoside use in CF patients and cumulative nephrotoxicity [3]. The continuing use of IV aminoglycosides in CF necessitates the development of strategies to reduce their negative renal impact.

Fosfomycin (1, 2-epoxy-propyl-phosphonic acid), originally isolated from *Streptomyces fradiae* [4], is now produced synthetically. It competes for the same renal binding sites as aminoglycosides. Animal models suggest that it might attenuate the nephro- [5, 6] and oto- [7] toxicity of aminoglycosides when co-administered. Furthermore, it has useful activity against Psa [8] and good lung tissue and biofilm penetration following IV administration [9].

We have previously demonstrated the efficacy of fosfomycin in CF pulmonary Psa exacerbations [10]. However, its potential renoprotective properties have not been evaluated in CF and to investigate this further, we conducted a prospective randomized crossover study of its use in combination with tobramycin and a second antibiotic (colomycin) in the treatment of Psa exacerbations.

MATERIALS AND METHODS

Study population

People with CF chronically infected with Psa experiencing two or more pulmonary exacerbations in the preceding 12 months and requiring admission to hospital formed the study population. Chronic Psa infection was defined as three or more positive sputum cultures within the previous 12 months [11]. An exacerbation was defined as the need for additional antibiotic treatment as indicated by a recent change in sputum volume or colour; increased cough; increased malaise, fatigue or lethargy; anorexia or weight loss; or radiographic changes or increased dyspnoea [12] associated with a decrease in FEV_1% from stable outpatient clinic baseline. Those with known intolerance to aminoglycosides, colomycin or fosfomycin had Psa isolates resistant to tobramycin or colomycin, a history of *Burkholderia cepacia (B. cepacia)* isolation in the preceding 12 months, significant haemoptysis or new radiographic changes, had received any aminoglycoside (IV or nebulized) therapy during the previous 3 months or any additional anti-pseudomonal antibiotic in the 2 weeks prior to admission, or did not experience a second exacerbation within 1 year were excluded (Figure 1). All refrained from vigorous physical exercise for 2 days prior to the study. Eighteen CF patients [mean (SD) age 21.8 (3.4) years, FEV_1 59.3 (15.1) % predicted, body mass index (BMI) 21.2 (2.4) kg/m^2, 10 males] completed the study.

Four had CFRD at enrolment and no new cases of diabetes were diagnosed in the remainder over the study period.

Written informed consent was obtained, and the study was approved by the local research ethics committee at the Liverpool Heart and Chest Hospital, UK.

Study design

At the first exacerbation, patients were randomized to receive 14 days of either IV tobramycin/colomycin (in line with the standard practice of using a minimum of two anti-pseudomonal antibiotics to treat pulmonary exacerbations in CF) or IV tobramycin/colomycin/fosfomycin. At the second exacerbation, patients received the alternative antibiotic combination.

IV tobramycin (80 mg/2 mL, Mayne Pharma Plc, UK) was given in two to three divided doses to achieve a trough level of <2.0 mg/L and a peak level of 6–10 mg/L (in keeping with recommended protocols). Levels were subsequently measured as needed to ensure therapeutic serum concentrations [mean (SD) daily dose of 7.6 (SD 0.8) mg/kg in the tobramycin/colomycin arm and 7.9 (0.9) mg/kg in the tobramycin/colomycin/fosfomycin arm; $P = 0.82$]. IV colistimethate sodium (Colomycin® injection, Forest Laboratories Ltd, UK) was given at a fixed dose of 2 MU three times a day (tid), and IV fosfomycin disodium (5 g powder for reconstitution, Idis Pharma, UK) at a fixed dose of 5 g tid.

Outcome measures

The primary efficacy endpoint was the antibiotic-related change from baseline to Day 14 in urinary levels of the proximal tubular enzymes *N*-acetyl-β-D-glucose-aminidase (NAG), alanine amino-peptidase (AAP) and the tubular protein β_2-microglobulin (β_2M). Renal indices also included serum creatinine (SCr) and magnesium (Mg^{2+}) assays, 24-h urine collection for

measurement of creatinine clearance (CCl) and total proteinuria/albuminuria.

Secondary efficacy endpoints were changes in spirometry (FEV_1, FVC), peripheral blood white cell count, C-reactive protein levels and the time to next exacerbation. Safety endpoints included adverse events (AEs) and toxic serum tobramycin concentrations.

Laboratory methods

Sputa at the beginning and end of the treatment were collected and processed using standard microbiological methods. Serum tobramycin concentrations were measured using TDxFLx assay (Abbott Laboratories). Serum and urine creatinine were measured by the Jaffe reaction and CCl calculated as urine volume × urine creatinine/SCr (expressed as mL/min).

NAG was measured using a commercial assay kit (PPR Diagnostics, London, UK) and the effects of varied urine concentration minimized by expressing NAG levels as a ratio of urine creatinine (i.e. units/mmol creatinine).

AAP (units/mmol creatinine) was measured using a modification of a procedure described by Jung and Scholz [13] with the change in absorbance at 410 nm calibrated by reference to a standard curve of known concentration of *p*-nitroaniline product.

After buffering to a pH of 7.0, β_2M (μg/mmol creatinine) was measured using a solid immune-radiometric assay (Euro/DPC, Llanberis, UK).

Urine total protein and albumin were quantified by a turbidimetric method in a Roche Clinical Chemistry analyser (Wako, Japan).

Statistical analysis

Based on published mean (SD) improvements in FEV_1 after IV antibiotic therapy of CF pulmonary exacerbations and our centre data of antibiotic-related changes in renal function, 130 patients would have been required to adequately power a study to detect a superior or non-inferior effect from the addition of fosfomycin comparing different antibiotic regimens. This number would have been more appropriate for a multicentre trial, which was not feasible; hence, we undertook a pragmatic single-centre pilot study, based on the resources available.

Baseline demographics were compared using a paired *t*-test or Chi-squared test. Pulmonary function, blood, sputum and urine test results on Days 0 and 14 are presented graphically as median and IQR. Within-group Days 0–14 changes were assessed using a paired *t*-test (parametric data) or the Wilcoxon signed rank test (non-parametric data). Treatment effects are presented as the mean [95% confidence interval (CI)] or median (IQR) difference between treatment-induced changes and analysed using a paired *t*-test or Wilcoxon signed rank test as appropriate. D'Agostino and Pearson's normality tests were used to assess data distribution throughout. Comparison of time to next exacerbation was conducted with Kaplan–Meier survival curves and a log-rank test. Results are presented as means (SD) unless otherwise stated.

RESULTS

All 18 patients completed both arms of the study (i.e. 36 treatment episodes). Table 1 shows baseline parameters at the start of each treatment. The mean (SD) interval between admissions was 4.6 months (2.9; range 1–11). Allowing for age change and baseline patient characteristics, the remainder of their therapy was otherwise identical in the two inpatient episodes.

Table 1. Baseline characteristics of lung function (FEV_1%, FVC), BMI, renal indices and renal markers at the start of each treatment

	TOB + COL ($n = 18$)	TOB + COL + FOM ($n = 18$)
FEV_1 % (predicted)	48.3 (13.1)	47.2 (13.8)
FVC % (predicted)	67.9 (10.0)	65.4 (8.1)
BMI (kg/m^2)	21.9 (2.6)	21.6 (2.7)
Blood urea (mmol/L)	4.4 (0.9)	4.2 (0.8)
SCr (μmol/L)	81.6 (10.1)	82.0 (11.8)
Serum Mg^{2+} (mmol/L)	0.90 (0.15)	0.83 (0.17)
CCl (mL/min)	84.3 (21.2)	81.4 (19.7)
24-h urine protein excretion (g)	0.08 (0.05)	0.07 (0.05)
Urinary albumin (mg/mmol)	1.6 (0.7)	1.8 (0.6)
Urinary NAG (IU/mmol)	0.28 (0.12–0.45)	0.26 (0.10–0.61)
Urinary AAP (IU/mmol)	0.95 (0.52)	0.84 (0.54)
Urinary β_2M (μg/mmol)	20.76 (11.90–30.25)	18.58 (12.00–28.50)
TOB daily dose (mg/kg)	7.6 (0.8)	7.9 (0.9)
COL daily dose (MU)	6 (–)	6 (–)
FOM daily dose (g)	N/A	15 (–)

Data are presented as mean (SD) or median (IQR) as appropriate.
COL, colomycin (colistimethate sodium); FOM, fosfomycin disodium; TOB, tobramycin; N/A, not applicable.

Changes in renal function

Urinary levels of the proximal tubular enzymes NAG and AAP after 14 days treatment with tobramycin/colomycin/fosfomycin were significantly lower than those recorded after treatment with tobramycin/colomycin alone. There were lower β_2M levels on triple therapy (Table 2 and Figure 2) although the difference did not reach statistical significance. The addition of fosfomycin to tobramycin/colomycin also attenuated the antibiotic-induced total urinary protein leak in a 24-h collection ($P < 0.0001$), but urinary albumin did not alter from baseline and did not differ between the two treatments.

Except for comparable improvements in CCl, none of the other renal indices changed significantly with either treatment. There were no instances of AKI by the KDIGO definition in either arm of the study.

Recovery from exacerbation

The mean 14-day improvements in all surrogate markers of exacerbation resolution were not statistically different between both treatments (Table 3).

Time to next exacerbation

The addition of fosfomycin did not impact on the time to next exacerbation requiring hospitalization. The interval between two consecutive hospital admissions was 4.2 (2.9) and 5.0 (1.3) months for patients treated initially with tobramycin/colomycin and tobramycin/colomycin/fosfomycin, respectively (χ^2 test = 0.89; HR = 1.52, 95% CI 0.59–4.41; $P = 0.35$).

Sputum microbiology

Thirty-one (86%) sputa were collected prior to treatment: all harboured Psa susceptible to tobramycin and colistin *in vitro* on standard disc diffusion testing. Psa eradication was not

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Table 2. Renal and urinary markers for each treatment group

Test	TOB + COL	TOB + COL + FOM	Difference between treatments (95% CI for difference)	P-value
Urea	0.68 (1.64)	0.62 (1.59)	−0.06 (−1.154 to 1.034)	0.91
Creatinine	4.33 (10.22)	2.17 (9.78)	−2.16 (−8.936 to 4.616)	0.52
CCl	17.28 (17.13)	19.89 (14.64)	2.61 (−8.128 to 13.348)	0.64
Mg^{2+}	−0.05 (0.02)	0.05 (0.19)	0.1 (−0.032 to 0.232)	0.13
Total proteinuria	0.27 (0.20)	0.04 (0.07)	−0.23 (−0.331 to −0.129)	0.0001
Albuminuria	0.19 (0.47)	0.25 (0.60)	0.06 (−0.313 to 0.417)	0.77
NAG	1.38 (0.63–4.38)	0.53 (0.24–1.36)	−1.24 (−3.313 to −0.447)	0.0028
AAP	30.1 (33.4)	11.8 (11.04)	−18.3 (−35.15 to −1.45)	0.03
β_2M	111.9 (19.0–179.80)	14.3 (0.16–118.8)	−57.50 (−0.1806 to 0.0126)	0.1297

Data are presented as mean (SD) or median (IQR) as appropriate. Unadjusted P-values are presented throughout. Change in renal indices and renal markers from the start of each treatment to Day 14 and comparison between treatments tobramycin/colomycin and tobramycin/colomycin/fosfomycin.
COL, colomycin (colistimethate sodium); FOM, fosfomycin disodium; TOB, tobramycin.

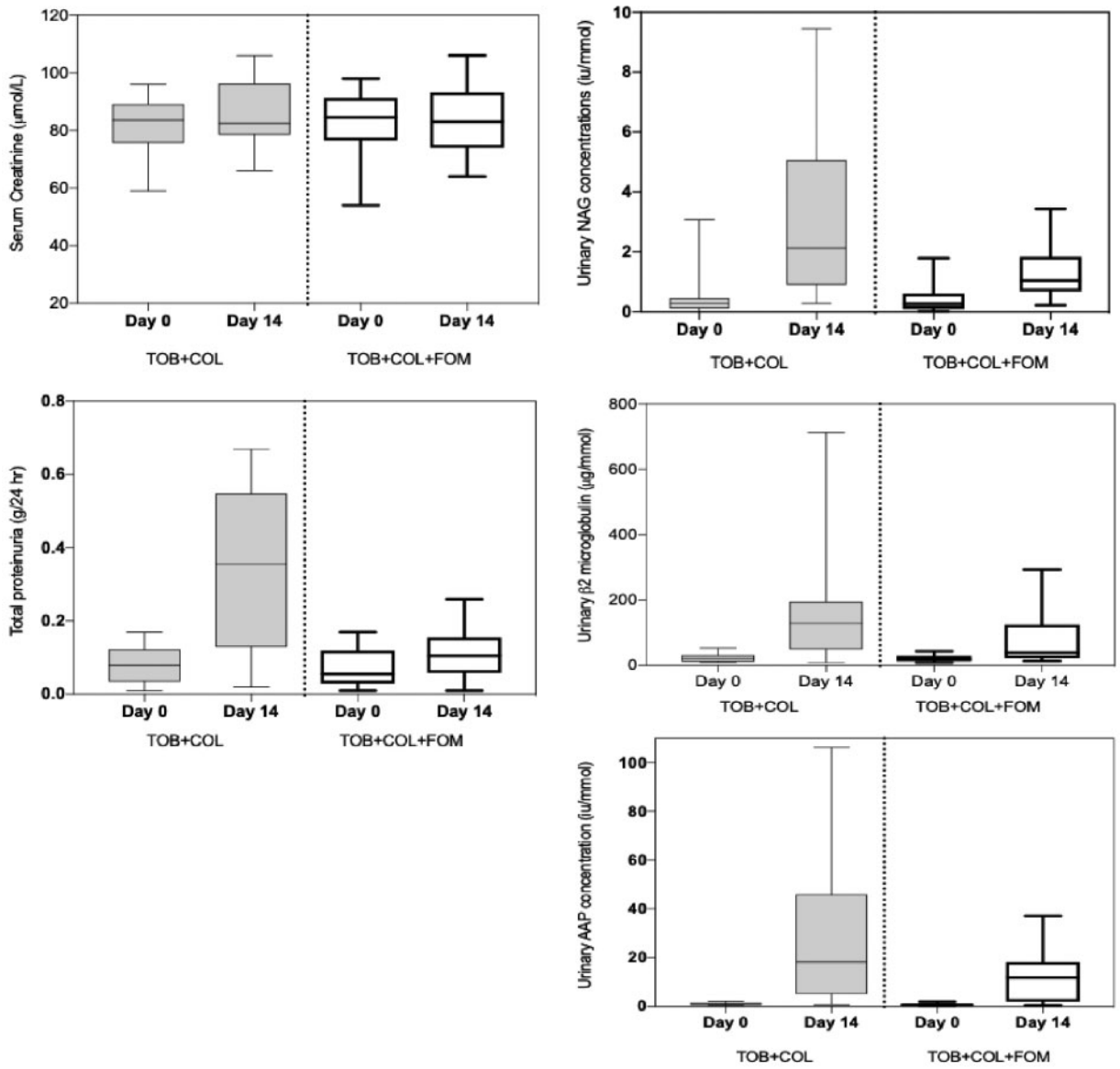

FIGURE 2: Change in renal markers from the start of (Day 0) to end (Day 14) of each treatment and comparison between treatments tobramycin/colomycin (TOB + COL) and tobramycin/colomycin/fosfomycin (TOB + COL + FOM).

Table 3. Surrogate markers of exacerbation resolution

Test	TOB + COL	TOB + COL + FOM	Mean difference between treatments (95% CI for difference)	P-value
FEV_1% (predicted)	+12.6 (8.0)	+11.8 (5.9)	−0.8 (−5.6 to 4.0)	0.73
FVC% (predicted)	+14.1 (7.7)	+15.9 (6.9)	1.8 (−3.15 to 6.75)	0.46
WCC	−2.9 (6.3)	−3.3 (4.1)	−0.4 (−4.0 to 3.2)	0.82
CRP	−27 (26.8)	−34 (30.0)	−7 (−26.3 to 12.3)	0.47

Data are presented as mean (SD). Change in lung function and inflammatory markers from the start of each treatment to Day 14 and comparison between treatments tobramycin/colomycin and tobramycin/colomycin/fosfomycin.
COL, colomycin (colistimethate sodium); CRP, C-reactive protein; FOM, fosfomycin disodium; TOB, tobramycin; WCC, white cell count.

Table 4. Number (%) of patients reporting treatment emergent AE

AE	TOB + COL	TOB + COL + FOM
Aggravated cough	3 (17)	3 (17)
Increased sputum	3 (17)	4 (22)
Acute bronchospasm/chest tightness	2 (11)	0 (0)
Sore throat/hoarseness	2 (11)	2 (11)
Reduced hearing	2 (11)	1 (11)
Tinnitus	2 (11)	0 (0)
Dizziness	3 (17)	1 (17)
Paraesthesia	1 (6)	1 (6)
Nausea	2 (11)	3 (17)
Diarrhoea	2 (11)	3 (17)
Treatment episodes with any reported AE	8 (44)	9 (50)

COL, colomycin; FOM, fosfomycin; TOB, tobramycin.

observed and treatment-emergent super-infection with new CF pathogens such as Burkholderia species, *Stenotrophomonas maltophilia* or other resistant Gram-negative bacteria was not recorded in any treatment episode.

Safety

AEs. There were no AEs warranting withdrawal of therapy. The incidence of AEs was comparable during both treatments (Table 4).

Serum tobramycin levels. Serum tobramycin assays were not statistically different throughout both treatments, and no toxic drug levels were recorded in either treatment arm.

DISCUSSION

Improving survival in CF has brought new complications as a consequence of a lifetime of necessarily aggressive treatment regimens. Renal disease in people with CF is becoming increasingly recognized as they accumulate exposure to nephrotoxic therapy, including aminoglycosides, which are a mainstay of treatment for Psa. Alternative strategies that spare aminoglycoside renal side-effects are necessary. For the first time, we have shown that fosfomycin may be useful in reducing the acute renal injury caused by aminoglycosides.

SCr on its own is a poor marker of renal function in CF. Due to their nutritional challenges, poor muscle mass and recurrent infections, many CF patients do not have normal or stable SCr levels, further complicating the use of readily available surrogate markers of renal function such as measured or estimated CCl [14]. Hence, more accurate indices of renal disease are necessary in CF. Since the primary nephrotoxic effect of aminoglycosides is on the proximal tubular epithelium [15], the release of cell-bound enzymes can then serve as sensitive markers of acute renal injury before conventional laboratory assays become deranged [14], and we therefore used these in our study. Indeed, these urine biomarkers have already been employed to study nephrotoxicity caused by immunosuppressive drugs [16] and antibiotics [17]. We used NAG, AAP and β_2M for this purpose, where elevated urinary levels indicate the site of primary tubular cell damage (AAP: brush border, NAG: tubular lysosomes) [18].

NAG is present in high concentrations in the proximal tubular cells, and its molecular weight (140 kDa) does not permit glomerular filtration. Raised urinary levels reflect proximal renal tubular dysfunction, and elevated urinary NAG excretion during IV aminoglycoside use in people with CF is well described [14, 19].

Elevated urinary AAP precedes increase in SCr in patients with renal toxicity [14]. The assay [13] can detect differences in enzymuria between various treatment regimens [17] and different severity groups of non-drug-related renal disease [20]. We chose AAP as a marker in this study because of its specificity to the proximal tubule cell, and changes in urinary AAP should therefore mirror those in NAG.

β_2M, a low molecular weight (12 kDa) plasma protein, is normally filtered at the glomerulus and completely reabsorbed by the proximal tubules where it is catabolized. Increased urinary β_2M leak has been found to correlate with altered renal function secondary to aminoglycoside exposure [21].

Other markers including neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule 1 are thought to be better associated with acute kidney insult [22, 23]. However, NGAL is also produced in response to damaged epithelial cells in the lung [24], confounding its utility in the CF condition. Another marker tissue inhibitor of metalloproteinase 2 (TIMP-2)·IGFBP7, which is a combination of urine TIMP-2 and insulin-like growth factor (IGF) binding protein 7 (IGFBP7), has been identified as a potential biomarker of AKI. Its urinary levels rise early after first antibiotic administration. However, given the significant heterogeneity among studies [25], caution has to be exercised about the utility and limitations of this biomarker in clinical practice, especially as there are no prior clinical studies validating its application in CF. Hence, we did not include these markers in our study.

In our study, although significant differences between the two regimens were shown with NAG and AAP, the impact of fosfomycin on β_2M levels was less dramatic.

There are several possible explanations for this. First, although NAG and AAP reflect tubular structural integrity, β_2M only acts as a surrogate marker of functional competency of the proximal tubular epithelium [26], but has traditionally been used in clinical practice to detect renal tubular damage [27]. It may well be that, unlike shorter drug challenges in animal studies [5, 6], continued exposure to tobramycin may have caused a functional change despite the relative structural preservation afforded in the fosfomycin arm. Secondly, β_2M degrades very rapidly at 37°C in the acidic (pH < 5.5) environment of the urinary bladder [28], and despite adjusting all samples to pH = 7 immediately after collection, it is possible that some degradation may have occurred thus confounding the urinary yield of β_2M and diluting any true protective effect with fosfomycin.

Nevertheless, there were lower urinary β_2M levels in the fosfomycin arm, and this is in keeping with the significantly lower total protein leak that was also noted. Since urine albumin levels were not different in both arms, this could not have been simply due to glomerular-related proteinuria.

Using these assays, we have demonstrated that incorporating fosfomycin into the antibiotic treatment of CF pulmonary exacerbations offers some protection against the immediate proximal tubular injury caused by tobramycin. Previously only described in animal models [5, 6], this is the first report to reproduce the nephroprotective properties of fosfomycin against aminoglycoside-induced tubulotoxicity in people with CF.

Furthermore, a number of studies have shown the utility of fosfomycin as an anti-Psa antibiotic in people with CF [10, 29, 30]. It is actively taken up by bacterial cells through two nutrient transport systems and inhibits the initial step in cell wall synthesis [31] present in various bacteria (including Psa). However, *in vitro* susceptibility testing for fosfomycin against Psa requires the presence of glucose-6-phosphate, which is not routinely incorporated into standard sensitivity testing agars such that sensitive strains may appear resistant [32], and indeed 28/36 Psa isolates in our cohort were reported to be fosfomycin-resistant.

Co-administration of fosfomycin may also enhance other antibiotics for a number of reasons. First, it can penetrate the negatively charged polysaccharide biofilm in the CF lung, which protects organisms from specific antibodies and other antibiotics. Secondly, a synergistic *in vitro* effect has been demonstrated in combination with ofloxacin against Psa growing in biofilm [33] and with ciprofloxacin against CF Psa isolates [34]. Thirdly, because it acts on different synthetic pathways, it reduces the potential for the development of cross resistance with other classes of antibiotics [35, 36]. Finally, it has excellent tolerability: there are no reports in the literature of the side effects of the IV formulation. Its potential to ameliorate aminoglycoside-associated nephrotoxicity in this study adds further support to its utility in the CF population.

In conclusion, people with CF chronically infected with Psa require repeated courses of IV anti-pseudomonal antibiotics and the frequent use of a limited selection of antibiotics encourages the development of resistance and increased toxicity including cumulative renal impairment. Fosfomycin is a unique bactericidal antibiotic, which expands the range of anti-pseudomonal therapy available for CF patients, and is safe and well tolerated with valuable renal-sparing properties. Whether this translates into clinically meaningful preservation of renal function needs to be ascertained in larger long-term studies.

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CONFLICT OF INTEREST STATEMENT

None declared.

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