Antimycobacterial Activity of Nonantibiotics Associated with the Polypharmacy of Cystic Fibrosis (CF) against Mycobacterium Abscessus

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Abstract

Background: Antimicrobial resistance (AMR) has rendered certain species of Mycobacterium difficult to treat clinically, particularly the nontuberculous Mycobacterium, Mycobacterium abscessus, in patients with cystic fibrosis (CF). Such patients are treated with several nonantibiotic medicines, which may have antimicrobial properties. Given the growing burden of AMR in M. abscessus, it is important to investigate the antimicrobial activity of all medicines used in the treatment of such patients. It was, therefore, the aim of this study to examine the antimicrobial activity of 10 nonantibiotic medicines used commonly in the treatment of CF. Methods: Antibiotic susceptibility studies were performed on human clinical isolates of M. abscessus (n = 16) including 11 smooth isolates, four rough isolates and one Reference isolate (NCTC 13031), against the following 10 nonantibiotic medicines: aspirin (850 μg/ml), chlorphenamine (400 μg/ml), Creon (4000 international units/ml), cyclizine (50 mg/ml), DNase (1 μg/ml), hypertonic saline (NaCl) 7% (w/v), ibuprofen (44.4 mg/ml), lansoprazole (300 μg/ml), paracetamol (10 mg/ml), and prednisolone (500 μg/ml). Results: Of the 10 nonantibiotic drugs investigated, inhibition of M. abscessus was noted with chlorphenamine (400 μg/ml), cyclizine (50 mg/ml), ibuprofen (44.4 mg/ml) and lansoprazole (300 μg/ml), with no activity associated with aspirin (850 μg/ml), Creon (4000 international units/ml), DNase (1 μg/ml), hypertonic saline (NaCl) 7% (w/v), paracetamol (10 mg/ml), and prednisolone (500 μg/ml). The minimum inhibitory concentration (MIC) of cyclizine to M. abscessus (n = 6) ranged from 8.0 to 12.5 μg/ml, with a mean MIC, MIC50, and MIC90 of 10.6, 10.0 and 12.5 μg/ml, respectively. Conclusion: This study identified that chlorphenamine, cyclizine, ibuprofen, and lansoprazole have in vitro antimycobacterial activity against clinical M. abscessus, isolated from patients with CF. Further studies should now examine potential antimicrobial synergy between these compounds and common conventional antimycobacterial antibiotics, including the macrolides and fluoroquinolones, to decide how best to exploit such positive interactions to reduce AMR burden and improve treatment regimens.

Keywords: Antibiotic resistance, cystic fibrosis, drug discovery, Mycobacterium abscessus, treatment

INTRODUCTION

The long-term use of several classes of antibiotic agents, including aminoglycosides, beta-lactams, fluoroquinolones, macrolides and polymyxin, for the prophylaxis, maintenance, and treatment of bacterial respiratory pathogens causing chronic chest infections in patients with cystic fibrosis (CF), has important consequences for the persistence of respiratory pathogens including the emerging nontuberculous mycobacteria (NTM), in particular, Mycobacterium abscessus. M. abscessus infection is CF has gradually emerged from early reports of its prevalence of 1.3% in 19841 to 4.9% in adult CF patients in the US in 2016,2 while the prevalence rates for total Mycobacterium spp. from the US Cystic Fibrosis Foundation Registry for 2016 were approximately 12%.3 This infection is associated with chronic persistence of the organism in the lower airways, leading to increased morbidity and mortality in some patients.4 Furthermore, the presence of...
this organism in patients’ sputum is usually a contraindication for lung transplantation. It is, therefore, important to have efficacious antibiotics available to treat the pulmonary disease associated with this organism. One worrying microbiological characteristic to emerge from studies to date is the relative antibiotic resistance of the NTM organisms in CF, where there is usually a high degree of antibiotic resistance to several classes of antibiotics.\(^5\)

Patients with CF routinely take a diverse variety of medicines (in addition to antibiotics) on a daily basis to treat various aspects of their disease. These include bronchodilators, mucolytic agents, nutritional supplements, pancreatic enzyme replacement therapy, proton pump inhibitors, and insulin if the patient has CF-related diabetes. To date, the antimicrobial activity of these drugs has not yet been investigated with the NTMs. Therefore, it was the aim of this study to examine the potential antimycobacterial activity of 10 nonantibiotic drugs that are commonly used in the treatment of CF.

**Methods**

**Description of Mycobacterium abscessus isolates used**

\(M.\) abscessus isolates (\(n = 16\)) were obtained from the HSC Microbiology Culture Respository, MicroARK (www.microark.com), housed at the Northern Ireland Public Health Laboratory, at Belfast City Hospital. These isolates consisted of 15 clinical isolates obtained from patients with CF and one Reference Strain (NCTC) obtained from the National Culture Type Collection, Public Health England (Colindale, London, UK). All isolates had been historically stored on slopes of Lowenstein–Jensen (LJ) medium in glass universal containers at ambient temperature. All isolates were recovered and passaged twice on Columbia agar base (Oxoid CM0331; Oxoid Ltd., Basingstoke, UK) supplemented with 5% (v/v) defibrinated horse blood, which was incubated at 37°C for 5 days, before employment in the current study.

**Antimicrobial sensitivity tests**

**Preparation of drug solutions**

Aspirin (850 ug/ml), chlorphenamine (400 ug/ml), Creon (4000 international units/ml), cyclizine (50 mg/ml), DNase (1 µg/ml), hypertonic saline (NaCl) 7% (w/v), ibuprofen (44.4 mg/ml), lansoprazole (300 ug/ml), paracetamol (10mg/ml), and prednisolone (500 ug/ml) were prepared.

**In vitro antimicrobial activity of nonantibiotic drugs**

Inoculum (0.5 McFarland standard) of each \(M.\) abscessus isolate was streaked on to the surface of individual Columbia Agar supplemented with 5% (v/v) defibrinated horse blood. The plates were labeled in sectors representing each drug plus appropriate controls. Duplicate 10 µl drops of each solution of drug was pipetted onto the media and left to dry. The plates were inverted and incubated at 37°C incubator for 5 days. Any observed zones of inhibition in the region of the test drug were recorded.

**Determination of the minimal inhibitory concentration of cyclizine**

Dilutions of cyclizine were prepared in 0.1% PS to make up concentrations of 1:1 (50 mg/L), (25 mg/L), (16.7 mg/L), (12.5 mg/L), (10 mg/L), (8.3 mg/L), (7.1 mg/L), (6.2 mg/L), (5.5 mg/L), and (5 mg/L). The inoculum was streaked on to Mueller Hinton agar. Cyclizine solution was pipetted on to the surface and left to dry before the plates were incubated at 37°C for 5 days. Any observed zones of inhibition in the region of the test drug were recorded.

**Results**

Of the 10 nonantibiotic drugs investigated, inhibition of \(M.\) abscessus was noted with chlorphenamine (400 ug/ml), cyclizine (50 mg/ml), ibuprofen (44.4 mg/ml), and lansoprazole (300 ug/ml), with no activity associated with aspirin (850 ug/ml), Creon (4000 international units/ml), DNase (1 µg/ml), hypertonic saline (NaCl) 7% (w/v), paracetamol (10 mg/ml), and prednisolone (500 ug/ml). The minimum inhibitory concentration (MIC) of cyclizine to \(M.\) abscessus \((n = 6)\) ranged from 8.0 to 12.5 µg/ml, with a mean MIC, \(MIC_{50}\), and \(MIC_{90}\) of 10.6, 10.0, and 12.5 µg/ml, respectively.

**Discussion**

The leading cause of mortality among the CF patients is lung failure resulting from the chronic presence of bacterial pathogens. The urgent need for novel antimicrobial drugs is merited by the increasing prevalence of resistant strains. The aim of this study was to examine the potential antimicrobial activity of nonantibiotic drugs that are commonly used in the treatment of CF. This study demonstrated antimycobacterial activity of four nonantibiotic medicines, commonly used in the treatment of patients with CF, namely chlorphenamine, cyclizine, ibuprofen and lansoprazole. All \(M.\) abscessus isolates tested were sensitive to these compounds. All other medicines examined did not show any antimycobacterial activity against any of the isolates tested. Given the increasing burden of antimicrobial resistance (AMR) globally, particularly with the mycobacteria, it is, therefore, important to explore all potential sources of antibacterial activity to strengthen our arsenal of availability antibiotics.

**Conclusion**

This small study identified that chlorphenamine, cyclizine, ibuprofen, and lansoprazole have in vitro antimycobacterial activity against clinical \(M.\) abscessus, isolated from patients with CF. Further studies should now examine potential antimicrobial synergy between these compounds and common conventional antimycobacterial antibiotics including the macrolides and fluoroquinolones, to decide how best to exploit such positive interactions to reduce AMR burden and to improve patient care.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES