International Journal of Antimicrobial Agents 46 (2015) 560–567

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

The combination of colistin and fosfomycin is synergistic against NDM-1-producing Enterobacteriaceae in in vitro pharmacokinetic/pharmacodynamic model experiments

Mahableshwar S. Albur*, Alan Noel, Karen Bowker, Alasdair MacGowan

Bristol Centre for Antimicrobial Research and Evaluation, Department of Medical Microbiology, North Bristol NHS Trust, Southmead Hospital, Westbury-on-Trym, Bristol BS10 5ND, UK

ARTICLE INFO

Article history:
Received 10 March 2015
Accepted 14 July 2015

Keywords:
Colistin
Fosfomycin
Combination
PK/PD model
Time–kill curve
NDM

ABSTRACT

The chemotherapeutic options against NDM-1-producing Enterobacteriaceae infections are limited and therefore combination therapy is gaining momentum to counter the secondary resistance and potential suboptimal efficacy of monotherapy. Colistin and fosfomycin are two separate classes of antimicrobial agents that act on bacterial cells by different mechanisms. Hence, there is a potential for both synergy and antagonism. In this study, the antibacterial effects (ABEs) of colistin and fosfomycin were systematically investigated by time–kill curve studies over 48 h as well as in an in vitro pharmacokinetic model over 96 h against six well characterised strains of NDM-1-producing Enterobacteriaceae (three isolates resistant and three susceptible to fosfomycin) at a standard inoculum of 10^6 CFU/mL. Clinically achievable free serum concentrations of colistin sulphate and fosfomycin were used. In a single-chamber in vitro model, peak/trough concentrations (C_{max}/C_{min}) and the half-life ($t_{1/2}$) for fosfomycin (250/40 mg/L and 2.7 h, respectively) and colistin sulphate (3.0/0.75 mg/L and 4 h, respectively) were used, along with a growth control. ABEs were measured by the decrease in viable bacterial counts (log kill), area under the bacterial kill curve (AUBKC) and population analysis profile (PAP). The combination of colistin and fosfomycin compared with either agent alone achieved increased bacterial killing and decreased the chance of emergence of resistance. Also, the ABEs of the combination were sustained for a longer duration and were evident both against fosfomycin-sensitive and -resistant strains. This study provides important information and support for the role of combination therapy against multidrug-resistant Gram-negative bacteria with limited therapeutic options.

1. Introduction

Antibiotic resistance has emerged as a major global health problem in the last decade [1]. The emergence of carbapenem-resistant Gram-negative bacteria, especially carbapenem-resistant Enterobacteriaceae, is a major concern [2]. At the same time, there is a shortage in terms of development of newer antibiotics against multidrug-resistant (MDR) bacteria [3,4]. This has led to the re-emergence of so-called 'older' antibiotics such as polymyxins and fosfomycin [5,6]. Excessive use of these agents in the treatment of carbapenem-resistant Gram-negative bacteria, especially of colistin, has inevitably led to secondary resistance and the emergence of pan-resistant bacterial isolates [7].

New Delhi metallo-β-lactamase (NDM) was first described in a clinical case as recently as in 2008, although subsequent retrospective analyses of stored cultures have identified the gene encoding bla_{NDM} in Enterobacteriaceae isolates from 2006 [8].

Since then, NDM carbapenemase-producing isolates have been reported from more than 40 countries around the world. Isolates carrying bla_{NDM} tend to carry other resistance-encoding genes and, therefore, chemotherapeutic options in the treatment of infections caused by NDM-producing isolates are very limited [9]. Combination therapy is becoming a popular strategy to combat the emerging resistance, with the additional benefits of antimicrobial synergy and breadth of antimicrobial spectrum [10]. Although antimicrobial combination therapy has been widely used in clinical practice for many decades in a variety of clinical scenarios, such as neutropenic sepsis, infective endocarditis and infections due to *Pseudomonas aeruginosa*, robust evidence is lacking especially when β-lactam agents are not part of the combination therapy regimen [11].

Colistin and fosfomycin have been used therapeutically for many decades despite limited data on their pharmacokinetics and pharmacodynamics until recently [12,13]. Both agents are bactericidal with different mechanisms of action at separate bacterial

* Corresponding author. Tel.: +44 117 323 5651; fax: +44 117 323 8513.
E-mail address: msalbur@hotmail.com (M.S. Albur).

http://dx.doi.org/10.1016/j.ijantimicag.2015.07.019
0924-8579/

targets, with colistin being active against the bacterial cell membrane and fosfomycin against the bacterial cell wall. Data on interaction between these two antimicrobial agents against NDM-1-producing Enterobacteriaceae are very limited.

The aim of this study was to evaluate the combination of colistin and fosfomycin against NDM-1-producing Enterobacteriaceae using a range of isolates with varying susceptibility to fosfomycin. The main focus of this study was to assess the antibacterial activity of single agents versus the combination as well as to assess the risk of emergence of secondary resistance by deploying clinically relevant dosage regimens of colistin and fosfomycin.

2. Methods and materials

2.1. Bacterial isolates

Six well characterised NDM-1-producing Enterobacteriaceae isolates (kindly donated by Prof. T.R. Walsh, Cardiff University, Cardiff, UK) were used. All six isolates were susceptible to colistin with a minimum inhibitory concentration (MIC) of <0.5 mg/L, and three of the six isolates were resistant to fosfomycin. MICs were determined by the Clinical and Laboratory Standards Institute (CSLI) broth microdilution method [14] using *Escherichia coli* ATCC 29212 and *P. aeruginosa* ATCC 27853 as controls.

2.2. Culture media

Cation-supplemented Mueller–Hinton broth (MHB 10%) (Oxoid Ltd., Basingstoke, UK) was used in all experiments. Freshly prepared glucose-6-phosphate (G6P) (Sigma–Aldrich, Gillingham, Dorset, UK) was added to the MHB medium at the start of the experiments and subsequently for any replenishment required to achieve a final concentration of 25 mg/L. Nutrient agar and chromogenic agar (both from Oxoid Ltd.) plates were used for subculture of aliquots from the model and matrices of the time–kill studies.

2.3. Antibiotics and adjuvants

For in vitro pharmacokinetic/pharmacodynamic (PK/PD) studies and MIC determination, colistin sulphate was purchased from Sigma–Aldrich and fosfomycin (Fosfocina®) was from Laboratorios ERN (Barcelona, Spain). G6P, an essential adjuvant necessary for fosfomycin antibacterial activity, was obtained from Sigma–Aldrich and was stored below −20 °C. The potency of the antibiotics and G6P was derived from the product literature and communication with the scientific advisers of the respective companies. All of the stock solutions of antibiotics and G6P were freshly prepared just before commencement of the experiments.

2.4. Time–kill curve studies

The bactericidal activity of fosfomycin (FOF) and colistin sulphate (CST) over 48 h was tested against overnight cultures of six well characterised NDM-1-producing Enterobacteriaceae isolates by time–kill curve analysis. Three of the isolates (isolates 35, 36 and 42) were susceptible to FOF and three (isolates 38, 39 and 40) were resistant. All of the six strains were susceptible to CST. The following concentrations reflecting peak (C_{max}), average and trough (C_{min}) concentrations, respectively, were used: 250, 20 and 5 mg/L for FOF; and 0.29, 0.16 and 0.1 mg/L for CST. A 4 × 4 drug exposure matrix of FOF with CST was used along with a growth control (GC) incubated at 35–37 °C. Subcultures were obtained from each tube at the time points 0, 1, 3, 6, 12, 24 and 48 h.

2.5. Pharmacokinetic profiles for the pharmacokinetic/pharmacodynamic model

The antibacterial effects (ABEs) of CST and FOF were tested against five well characterised NDM-1-producing Enterobacteriaceae isolates in a single-chamber in vitro PK/PD model over 96 h. Three of the isolates (isolates 35, 36 and 42) were susceptible to FOF and two (isolates 39 and 40) were resistant. C_{max}/C_{min} values of 3.0/0.75 mg/L and 250/40 mg/L, respectively, for CST and FOF were simulated in the model. These concentrations correspond to the PK profiles of free drug serum concentrations achieved in 8-hourly intravenous dosage regimens of 2 MU for colistin methanesulfonate and 50 mg/kg for FOF. They were derived from previously published studies from leading researchers in corresponding field for CST and FOF [13,15]. The concentration of G6P was maintained at 25 mg/L in the MHB medium throughout the duration of the experiments.

Owing to lack of facilities to measure CST and FOF levels in our laboratory, extensive preliminary studies were performed using ganciclovir (surrogate for CST) and flucytosine (surrogate for FOF). These two non-bacterial agents were chosen because the high-performance liquid chromatography (HPLC) turnaround time for their results is quick and the ranges are similar to the corresponding antibiotic that we were using in the model, which facilitated for us to modify the flow/concentration in a timely fashion. To adjust for the differences in half-life ($t_{1/2}$) of the two drugs (CST $t_{1/2}$ = 4 h and FOF $t_{1/2}$ = 2.7 h), an additional reservoir with a dosing chamber was added to generate an accurate and consistent flow rate in the model to achieve the desired concentrations. Once the flow rates of the model were finalised, repeat experiments were conducted on at least three occasions to verify the accuracy and consistency of the concentrations achieved.

2.6. In vitro model

A FerMac 310 Fermentation System (ElectroLab, Tewkesbury, UK), single-compartment in vitro PK/PD model was used to examine the microbiological response and the emergence of resistance to CST, FOF and their combination over 96 h. The apparatus, which has been described previously, consists of a single central chamber connected to a reservoir containing broth [16]. The central chamber with a capacity of 360 mL is connected to a collecting vessel for overflow. The contents of the central chamber were diluted with broth using a peristaltic pump (Ismatec®; Cole–Parmer, London, UK) at a defined flow rate. The temperature was maintained at 37 °C throughout and the broth in the central chamber was agitated by a magnetic stirrer. MHB was used in the model and an overnight culture of bacterial isolates was inoculated into the chamber in order to achieve an initial inoculum of 10^6 CFU/mL. Freshly prepared stock solutions of CST, FOF and G6P were instilled into the model as a bolus to achieve 3.0, 250 and 25 mg/L, respectively, at the start of the experiments, and subsequent bolus doses were given every 8 h until 96 h.

2.7. Antibacterial effects

Using an initial bacterial inoculum of 10^6 CFU/mL, ABEs were measured as previously described [17]. Samples were taken from the central chamber of the PK/PD model at the following time points: 0, 1, 2, 4, 8, 12, 24, 36, 48, 60, 72, 84 and 96 h. Samples were subcultured onto nutrient agar and chromogenic agar for detection of viable bacterial counts. Bacteria were quantified using a spiral plater (Don Whitley Spiral Systems, Shipley, UK). The minimum level of detection of viable bacteria was 10^2 CFU. Aliquots were collected for population analysis profile (PAP) studies.