In-vitro activity of the combination of colistin and rifampicin against multidrug-resistant strains of Acinetobacter baumannii

*Corresponding author.

J Antimicrob Chemother 1998; 41:494–495

G. M. Hoge*, J. G. Barr and C. H. Webb

Department of Bacteriology, The Royal Group of Hospitals and Dental Hospital Health and Social Services Trust, Belfast BT12 6BA, UK

*Corresponding author. Tel: +44-1232-240503; Fax: +44-1232-311416.

Sir,

Acinetobacter spp. are ubiquitous organisms that are commonly found in the environment as free-living saprophytes. They are also widely distributed in the hospital environment and are present as commensals on the skin of healthy people. The latter is a likely source of many of the hospital outbreaks of infection caused by these bacteria, particularly in intensive care units (ICUs).

Successful treatment of patients with infections caused by Acinetobacter spp. is compromised by the resistance of most isolates to multiple antibiotics. Synergy between carbenicillin and aminoglycosides has been reported, but there is little published evidence of the in-vitro activities of other antibiotic combinations against strains of these species. In this study, we have evaluated the activity of the combination of rifampicin and colistin sulphate against 13 multiresistant clinical isolates of Acinetobacter baumannii.

A. baumannii strains 1–9 were isolated from blood cultures obtained during 1994/5. Isolates 10 and 11 were recovered from the blood and a central line tip, respectively, of two patients during a 1995 outbreak in the ICU caused by multiresistant strains of A. baumannii, and strains 12 and 13 were isolated from the blood of ICU patients in the post-outbreak period. Identification of the isolates was based on the characteristic colonial morphology on blood agar (0.5–2 mm in diameter, translucent to opaque, convex, and entire) and the lack of cytochrome oxidase activity; the strains were speciated with the API 20 NE System (bioMérieux, Marcy-l’Étoile, France).

Susceptibility to a wide range of antibiotics, including colistin sulphate and rifampicin, was determined by the disc diffusion method. The activity of the combination of rifampicin and colistin was evaluated using the checkerboard microbroth dilution method. The medium used was cation-adjusted Mueller–Hinton broth and the MIC of each antibiotic for each isolate was determined beforehand to facilitate the range of concentrations used in the synergy studies. The fractional inhibitory concentration index (FICI) for each isolate was calculated according to the following equation:

\[
\text{FICI} = \frac{\text{MIC of colistin alone}}{\text{MIC of rifampicin in combination}} + \frac{\text{MIC of rifampicin alone}}{\text{MIC of colistin in combination}} - 1
\]

The results were interpreted as follows: synergy, if FICI ≤ 0.5; indifference, if 0.75 < FICI < 2; antagonism, if FICI ≥ 2.

All 13 A. baumannii isolates were resistant to azlocillin, piperacillin, piperacillin/tazobactam, cefuroxime, cefotaxime, ceftazidime, aztreonam, gentamicin, amikacin, netilmicin, and ciprofloxacin and moderately resistant (MICs, 8 mg/L) to imipenem (resistance breakpoint, >8 mg/L) and meropenem (resistance breakpoint, >8 mg/L). Nine strains were resistant to rifampicin (MICs, 4–8 mg/L; resistance breakpoint, >2 mg/L), two were moderately resistant (MICs, 2 mg/L) and two were susceptible (MICs, 1 mg/L).

The combination of colistin and rifampicin was synergic against 11 isolates (FICIs, 0.07–0.63) and indifferent against two (FICIs, 1 and 1.13, respectively); antagonism was not observed. Although only two isolates were categorized as susceptible to rifampicin, this agent exhibited synergy with colistin against 11 isolates. It has previously been demonstrated that rifampicin combined with other antimicrobials may be synergic against some multiresistant Gram-negative bacteria. Synergy studies are therefore recommended when the use of this compound in combination with other agents is contemplated.

The A. baumannii isolates investigated in the present study had very similar multiple resistance patterns which effectively limited therapy to colistin, with the potential for the activity of this drug to be enhanced by adding rifampicin. However, synergy between these agents could not be predicted on the basis of the MICs of each drug alone and must be confirmed for individual clinical isolates before the combination is used.

In conclusion, we have demonstrated the theoretical value of the combination of colistin and rifampicin as treatment for patients with infections caused by A. baumannii. However, the potential toxicity of colistin (less so with colistin sulphomethate than with the sulphate formulation) will limit its use to those patients with infections caused by strains resistant to alternative, less toxic agents.

Correspondence

A newly recognized acetylated metabolite of arbekacin in arbekacin-resistant strains of methicillin-resistant Staphylococcus aureus

_J Antimicrob Chemother_ 1998; 41:495–497

Shigeru Fujimura*, Yutaka Tokue*, Hiroshi Takahashi*, Toshihiro Nukiwa**, Kanekicho Hisamichi*, Takeshi Mikami† and Akira Watanabe*

*Department of Respiratory Oncology and Molecular Medicine, Institute of Development, Aging and Cancer, Tohoku University, Sendai 980; †First Departments of Medicinal Chemistry and ‡Department of Microbiology, Tohoku College of Pharmacy, Sendai 981, Japan

*Corresponding address: School of Nursing, Miyagi University, 1-1, Gakuen, Taiwa-cho, Miyagi pref., 981-32, Japan. Tel and Fax: +81-22-377-8289.

Sir,

Arbekacin, 1-N-(S)-4-amino-2-hydroxybutyryldibekacin, is a novel, semi-synthetic aminoglycoside which was synthesized in Japan in 1973.1 It has a broad spectrum of activity, including bacteria resistant to kanamycin, gentamicin and tobramycin, principally because it is not inactivated by aminoglycoside-modifying enzymes. Arbekacin has been used as treatment of patients with infections caused by methicillin-resistant _Staphylococcus aureus_ (MRSA) in Japan since 1990 and the isolation of MRSA strains resistant to this antibiotic was first reported in 1992.2 The basis of this resistance has been shown to be the bifunctional aminoglycoside-modifying enzyme, aminoglycoside phosphotransferase (APH(2))–aminoglycoside acetyltransferase (AAC(6′)), which simultaneously phosphorylates and acetylates.3 We describe here a newly recognized metabolite of arbekacin in arbekacin-resistant strains of MRSA.

The six MRSA strains studied were clinical isolates recovered from the wounds of six patients. Four of the strains (1, 3, 4 and 5) were resistant to arbekacin (MICs > 6.25 mg/L) and one (6) was susceptible (MIC = 0.78 mg/L). The sixth strain (2) was initially susceptible to arbekacin, but developed resistance (MIC = 25 mg/L) following serial passage in the presence of the antibiotic at a concentration equivalent to 0.5 × MIC according to a method described previously.4

A suspension of each strain in 5 mL of broth containing 5 mg of arbekacin was adjusted to give a turbidity equivalent to that of a no. 1 McFarland standard and incubated for 24 h at 37°C. The suspension was then passed through a 0.22 µm Millipore filter. The metabolites in the cell-free filtrate were separated by Sep-pak (Waters) column chromatography and the eluates were concentrated in vacuo and analysed by various techniques. NMR (1H and 13C) spectra were obtained with a JNS-GSX 400 spectrometer (Jeol, Tokyo, Japan), with sodium 2,2-dimethyl-1,2-silapentane-5-sulphonate (DSS) as an internal standard. Mass spectrometry was performed with a JMS-DX 303/JMA-DA 5000 System high-resolution mass spectrometer (Jeol) and Fourier transform infrared (FT-IR) spectra were obtained with a DR-81 FT-IR spectrometer (Jasco, Tokyo, Japan).

Crude enzyme preparations were obtained by incubating a suspension of each strain in 250 mL of 1% Bacto Tryptone broth (Difco, West Molsley, UK) containing 0.5% NaCl and 0.5% yeast extract at 37°C for 8 h on a rotary shaker. The suspension was centrifuged at 3000 rpm for 20 min at 4°C and the supernatant removed. The cell deposit was then washed with 0.05 M Tris–HCl (pH 7.5) and resuspended in 5 mL of 0.05 M Tris–HCl 10 mM MgCl2 buffer (pH 7.8). Lysostaphin (Sigma, St Louis, MO, USA), at a concentration of 25 mg/L, was added to the suspension which was incubated for 30 min at 37°C. The cells were disrupted by sonication and the suspension was centrifuged at 30,000g for 30 min; the supernatant represented the crude enzyme preparation.5 The ratio of inactivation of arbekacin was determined by the method of Haas & Dowding.5 Briefly, a mixture comprising 0.05 mL of arbekacin (at a concentration of 400 mg/L), 0.05 mL of 0.02 M ATP (Sigma), 0.05 mL of 0.5 mM acetyl CoA (Sigma), 0.05 mL.

---

Correspondence

References


