including human isolates with varying susceptibilities to penicillin (Table). For the strain used in the infection model the nisin MIC was 0.06 mg/L.

An intravenous two-treatment nisin regimen of ≥0.16 mg/kg/treatment (total dose ≥0.31 mg/kg) protected 100% of the mice and 80% were protected by 0.63 mg/kg in a single iv dose or in two sc treatments (Table). Vancomycin at 1.25 mg/kg/dose protected 80% of the mice.

Five minutes after an iv dose of 20 mg/kg, the nisin concentration in serum was approximately 50 mg/L and declined with an apparent half-life of elimination of 0.9 h. Nisin was not detectable after 3 h.

Nisin showed excellent in-vitro activity against clinical isolates of S. pneumoniae, including penicillin-resistant and -intermediate strains, some of which were resistant to the other antimicrobial agents tested, with the exception of vancomycin. Nisin is rapidly bactericidal for S. pneumoniae clinical isolates at concentrations ≤1 mg/L (A. Tomasz, personal communication) and against staphylococci and enterococci at concentrations near the MIC (unpublished data). Rapid bactericidal activity against susceptible organisms is a characteristic of cationic peptides and is related to their mechanism of action.1

Although nisin and vancomycin had similar MIC for S. pneumoniae, including the strain used in the infection model, nisin was 8–16 times more active in a two-dose iv regimen in mice. As nisin had a serum half-life in mice of only 0.9 h, it is not surprising that two doses were needed for optimal activity, as previously reported for vancomycin.3 Low blood and tissue levels of nisin appear to be sufficient to prevent the death of mice infected with S. pneumoniae Felton, perhaps because of nisin’s rapid bactericidal action.

Vancomycin is an alternative therapy for serious infections with multiply resistant S. pneumoniae and the first-line treatment for methicillin-resistant staphylococcal infections. Resistance to vancomycin in enterococci is increasing and there have been recent reports of S. aureus with intermediate resistance to the drug.4 New therapies for Gram-positive infections are needed. Eukaryotic and synthetic antimicrobial peptides are under investigation as topical agents and as potential treatments for Gram-negative sepsis.1 Our data suggest that there may be a role for peptides such as nisin in the treatment of systemic disease. In addition to nisin itself, we are investigating variant molecules, produced by genetic engineering, which may have improved characteristics such as greater solubility or a better pharmacokinetic profile.

Acknowledgement

Some of these data were presented at the Ninety-Seventh General Meeting of The American Society for Microbiology, May 4–8, 1997, Miami, FL, USA.

References


An isocratic high performance liquid chromatography (HPLC) assay for moxifloxacin, a new 8-methoxyquinolone


Bristol Centre for Antimicrobial Research & Evaluation and the University of Bristol, Department of Medical Microbiology, Southmead Hospital, Westbury-on-Trym, Bristol, BS10 5NB, UK

*Corresponding author: Tel: +44-117-9595654; Fax: +44-117-9593217.

Sir,

Moxifloxacin (BAY 12-8039) is a new 8-methoxyquinolone with enhanced anti-Gram-positive activity in vitro compared with ciprofloxacin and ofloxacin.1,2 In-vitro sensitivity results have thus far indicated a potential clinical role in the treatment of community-acquired respiratory tract infection.3,4 The mean peak plasma concentration (T_max = 1 h) after a 600 mg oral dose has been reported to be around 4 mg/L, with a steady state trough concentration of 1.0 mg/L. A standard dose of 400 mg once daily has been proposed for moxifloxacin.5

As with all new drug therapies, it is important to have a reliable assay to optimize therapy, study clinical pharmacokinetics and investigate any possible drug interactions. We have therefore developed a simple HPLC assay. The stationary phase was Spherisorb S5 C8 in a stainless steel column, 100 × 4.6 mm heated to 50°C (Waters Ltd, Watford, UK). The mobile phase was 0.16% ortho-phosphoric acid, adjusted to pH 3 with tetra-butylammonium hydroxide; 50 mL of acetonitrile was...
Correspondence

added to the 1 L solution after the pH adjustment. The flow rate was 1.5 mL/min. Detection was by fluorescence (excitation wavelength 290 nm emission wavelength 500 nm: Perkin Elmer model LC240 detector, Beaconsfield, UK). Serum samples were mixed 50:50 with methanol, allowed to stand for 5 min and centrifuged at 25,000g for 5 min. The supernatant was mixed 50:50 with water and centrifuged again. Fifty microlitres of the supernatant was injected.

A chromatogram of moxifloxacin, and moxifloxacin separated from a mix of seven fluoroquinolones, is shown in the Figure. The reproducibility of the assay, expressed as the percentage coefficient of variation (% CV), was <8% after the repeat assay (n = 6) of aqueous and serum samples spiked with 1.0 mg/L moxifloxacin. The detection limit (in serum), defined as the moxifloxacin concentration equivalent to a peak three times the height of the base-line noise, was 0.002 mg/L. Linearity and serum recovery were investigated by assaying aqueous and serum specimens containing 0, 0.5, 0.75, 1, 2 and 4 mg/L moxifloxacin. The moxifloxacin peak height was plotted against drug concentration and regression analysis performed. The correlation between drug concentration and peak height was good for the aqueous and serum moxifloxacin samples ($r=0.999$ and 0.998, respectively). The percentage serum recovery (serum peak height/aqueous peak height $\times 100$) approached 100% at each drug concentration assayed. The accuracy of the assay was investigated by the assay of serum samples containing 0.1, 0.9 or 3.5 mg/L moxifloxacin using a single standard of 4 mg/L. The accuracy, expressed as the percentage error ([measured concentration–target concentration]/target concentration $\times 100$) was 0.0% for the 0.1 mg/L sample, 4.4% for the 0.9 mg/L sample and 0.3% for the 3.5 mg/L sample. The assay was specific. Following the assay of 22 commonly used antibiotics (including other fluoroquinolones) and antifungals and 24 patient samples (containing unknown drugs) no chromatographic peaks were seen that could potentially interfere with that of moxifloxacin.

This simple, accurate, reproducible assay for moxifloxacin is ideal for the clinical laboratory for drug monitoring and pharmacokinetic studies. It can readily be employed using equipment common to the assay of other fluoroquinolones.

Acknowledgement

Some of the data have previously been presented as a poster at the Thirty-Seventh Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, Canada, 1997 (Abstract F150).

References


Figure. Chromatogram of (a) moxifloxacin (1.0 mg/L) in serum (retention time approximately 6 min) and (b) moxifloxacin separated from a mixture of seven other fluoroquinolones (ciprofloxacin, ofloxacin, levofloxacin, norfloxacin, lomefloxacin, enoxacin and sparfl oxacin).