In vitro susceptibility testing of BMS-284756 by the BSAC standardized disc testing method

J. M. Andrews* and R. Wise

Department of Microbiology, City Hospital NHS Trust, Dudley Road, Birmingham B18 7QH, UK

*Corresponding author. Tel: +44-121-507-5693; Fax: +44-121-551-7763.

Sir,

BMS-284756 (BMS; T-3811ME) is a novel compound, lacking the 6-position fluorne typical of existing fluoroquinolones, which has been shown to have broad-spectrum activity against both Gram-negative and -positive pathogens (T. M. A. Weller, J. M. Andrews, G. Jevons & R. Wise, unpublished results). In this communication we describe a method of disc susceptibility testing that has been developed following the recommendations of the BSAC.1

A total of 763 strains, comprising 80 Escherichia coli, 50 each of the following: Klebsiella species, Proteus mirabilis, Proteus vulgaris, Staphylococcus aureus (including 20 MRSA), coagulase-negative staphylococci (including four methicillin-resistant strains), 20 each of the following: Providencia species, Serratia species, Acinetobacter species, 19 each of groups A and B streptococci, 10 Neisseria meningitidis, 37 Neisseria gonorrhoeae, 100 Streptococcus pneumoniae [including two mutants with par(C) Ser-79→Tyr and either a gyr(A) or Glu-85→Lys substitutions; one clinical strain with a par(C) Ser-79→Phe, par(E) Ile-460→Val and gyr(A) Ser-81→Tyr substitutions together with an efflux phenotype; one recent fluoroquinolone-resistant clinical isolate], and 94 each of Moraxella catarrhalis and Haemophilus influenzae were studied. Included in these strains were the appropriate NCTC and ATCC control strains. Briefly, the method employed IsoSensitest agar (Oxoid, Basingstoke, UK) prepared following the manufacturer’s instructions, supplemented as follows; P. mirabilis MIC determinations 50 mg/L p-nitrophenyl glycerol (PNPG; Sigma Diagnostics, Poole, UK) and for fastidious organisms 5% defibrinated horse blood (Tissue Culture Services, Clayden, UK) and 20 mg/L β-nicotinamide adenine dinucleotide (NAD; Sigma). An inoculum equivalent to semi-confluent growth was used for disc testing and an inoculum of 10^4 cfu/spot for MIC determinations. Incubation was at 35–37°C for 18–20 h in air, except for fastidious organisms, where incubation was in an atmosphere enriched with 4–6% CO_2 in air. Discs were prepared ‘in-house’ as described previously,2 at contents of 1, 2 and 5 μg. Zone diameters were measured manually using a ruler and zone diameters were recorded in millimetres. The MIC was defined as the lowest concentration of BMS inhibiting the visual growth of the organism after overnight incubation.

Pharmacokinetic (C_{max} of c. 6 mg/L following an oral dose of 400 mg and a terminal half-life of 13–17 h) and MIC distribution data for the strains tested, lacking a mechanism of resistance for all of the genera studied, was applied to the BSAC formula,3 and an MIC breakpoint (MIC BP) of 0.5 mg/L was calculated. Zone diameter data were analysed by scattergram plot and the degree of false susceptible and false resistant rates determined.

In the first instance, data were combined for all the bacterial species and each of the disc contents studied. Unacceptably large zones for the susceptible population were observed for the 5 μg disc (c. 48 mm; data not shown). For a 2 μg disc using a zone diameter breakpoint (ZDB) of 20 mm, a false susceptible rate of 1.2% (nine of 763 strains) and false resistant rate of 0.9% (seven of 763 strains) were calculated (data not shown). All of the strains where false interpretation was seen had MICs straddling the MIC BP, and they comprised mainly Enterobacteriaceae and Acinetobacter spp. (13 of 16 strains). Of greater importance were the three strains of S. pneumoniae with known mechanisms of resistance to quinolone antibiotics, which were considered susceptible by disc testing, but resistant by MIC determination (MICs of 1 mg/L to BMS). The zones of inhibition for the strains of N. gonorrhoeae known to have reduced susceptibility to quinolones ranged from 20 to 37 mm when a 2 μg BMS disc was tested. The detection of low-level resistance to quinolones in haemophili and Neisseria has proved problematic, and the BSAC recommendations indicate that the detection of reduced susceptibility is best achieved using the less potent quinolone nalidixic acid.1 As BMS has been developed primarily to target respiratory tract pathogens, the data for a 1 μg disc were separated to include results for the respiratory pathogens (S. pneumoniae, M. catarrhalis and H. influenzae) and analysed further (Figure). Using a disc content of 1 μg, 90% of strains had zone diameters between 28 and 40 mm. No false susceptible and only one strain interpreted as falsely resistant was observed using a ZDB of 20 mm. Using these criteria the four strains with reduced susceptibility to quinolones were considered resistant. Mean zone diameters (mm) and standard deviations (s.d.) for the control
Correspondence

Figure. Scattergram plot of BMS MIC versus zone diameter using a 1 μg BMS disc content for 288 respiratory pathogens (100 S. pneumoniae and 94 each of H. influenzae and M. catarrhalis).

strains using a 2 μg disc were 33.6 (1.2), 32.5 (1.1), 35.6 (1.4) and 32.3 (1.7), respectively, for E. coli NCTC 10418 and ATCC 25922, S. aureus NCTC 6571 and ATCC 25923. Using a 1 μg disc, mean zone diameter (S.D.) of 37.4 (1.3), 33.8 (1.7), 28.6 (1.4) and 42.9 (1.3), respectively, were observed for H. influenzae NCTC 11931 and ATCC 49247, S. pneumoniae ATCC 49619 and N. gonorrhoeae ATCC 49226.

These data indicate that, by BSAC methodology, using an MIC BP of 0.5 mg/L for pneumococci, streptococci and haemophili (this tentative breakpoint may be increased to 1 mg/L for Enterobacteriaceae and staphylococci when clinical response data have been reviewed) and a disc content of 2 μg is appropriate for all of the bacterial species studied except the common respiratory pathogens, where a disc content of 1 μg gave more reliable results.

References

