ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 2009, p. 5181–5184 Vol. 53, No. 12
0066-4804/09/$12.00 doi:10.1128/AAC.00118-09

Bacterial Strain-to-Strain Variation in Pharmacodynamic Index Magnitude, a Hitherto Unconsidered Factor in Establishing Antibiotic Clinical Breakpoints[▽]

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Received 27 January 2009/Returned for modification 9 July 2009/Accepted 24 September 2009

Antibiotic pharmacodynamic modeling allows variations in pathogen susceptibility and human pharmacokinetics to be accounted for when considering antibiotic doses, potential bacterial pathogen targets for therapy, and clinical susceptibility breakpoints. Variation in the pharmacodynamic index (area-under-the-concentration curve to 24 h [AUC_{24}]/MIC; maximum serum concentration of drug in the serum/MIC; time the serum concentration remains higher than the MIC [*T* > MIC]) is not usually considered. In an in vitro pharmacokinetic model of infection using a dose-ranging design, we established the relationship between AUC_{24}/MIC and the antibacterial effect for moxifloxacin against 10 strains of *Staphylococcus aureus*. The distributions of AUC_{24}/MIC targets for 24-h bacteriostatic effect and 1-log, 2-log, and 3-log drops in bacterial counts were used to calculate potential clinical breakpoint values, and these were compared with those obtained by the more conventional approach of taking a single AUC_{24}/MIC target. Consideration of the AUC_{24}/MIC as a distribution rather than a single value resulted in a lower clinical breakpoint.

The present antibacterial pharmacodynamic paradigm to establish antibiotic dosing, target pathogens for treatment, and establish possible clinical susceptibility breakpoints (S) was established by Drusano et al. (6). It depends, first, on using preclinical data from infection models to determine the dominant pharmacodynamic index (area under the concentration-time curve [AUC]/MIC; maximum concentration of drug in serum/MIC; time > MIC) for an agent. Second, knowledge of the relationship between this index and pathogen killing and/or animal survival, together with data on the MIC distribution of potential pathogens and the pharmacokinetics of the drug in healthy human volunteers or infected patients, is used in mathematical simulations. Mathematical modeling techniques are used to incorporate variations in the drug pharmacokinetics so that the proportion of a simulated cohort of patients who reach a predefined pharmacodynamic index target can be determined for each MIC of a potential infecting pathogen. The pharmacodynamic index target chosen is critical to this process, and for fluoroquinolones, a free-drug AUC_{24}/MIC ratio of around 30 is associated with a 1- to 2-log reduction in bacterial counts of *Streptococcus pneumoniae* after 24-h exposure in animal models and an 80% animal survival rate. In humans, a similar free-drug 24-h AUC (AUC_{24})/MIC ratio has been associated with improved microbiological responses in pneumococcal infection (1, 2). Although mathematical tools such as Monte Carlo simulations have been employed to model pharmacokinetic variability, it is also clear that there is variability in the pharmacodynamic index target—some variability is related to differences between species (10), some to differences between different bacterial strains within species (9), and some to experimental variation.

We used an in vitro pharmacokinetic model to determine strain-to-strain differences in the relationship between AUC_{24}/MIC and antibacterial effect for an exemplar fluoroquinolone-moxifloxacin treatment against *Staphylococcus aureus* strains. We then, for the first time, modeled the effects of strain-to-strain variation and experimental variation in the pharmacodynamic index on determination of a clinical susceptibility breakpoint for an antimicrobial.

(These data were presented at the 48th Interscience Conference on Antimicrobial Agents and Chemotherapy/46th Annual Meeting of the Infectious Diseases Society of America, Washington, DC, 25 to 28 October 2008 [11].)

MATERIALS AND METHODS

Microorganisms. Ten clinical strains of *Staphylococcus aureus* isolated in 2007 in the Department of Medical Microbiology, Southmead Hospital, Bristol, United Kingdom, were used. The strains were selected to include a range of MICs but with a bias toward strains having MICs close to the MIC_{50} of the wild-type population for moxifloxacin, which is 0.03 to 0.06 mg/liter. Testing of susceptibility to moxifloxacin was determined by broth dilution methods as described by the Clinical and Laboratory Standards Institute (CLSI) (5), except that intermediate concentrations (between those of the standard doubling dilution series) were tested.

In vitro pharmacokinetic model. A New Brunswick (Hatfield, Hertfordshire, England) Bioflo 1000 in vitro pharmacokinetic model was used to simulate serum free-drug concentrations of moxifloxacin when administered orally. The apparatus, which has been described before, consists of a single central chamber connected to a dosing chamber, which is in turn attached to a reservoir containing broth. The central chamber (360 ml) is connected to a collecting vessel for overflow. The contents of the dosing chamber and central chamber were diluted with broth by using a peristaltic pump (Ismatec, Cole Palmer, England) at a flow rate of 66 ml/h. The temperature was maintained at 37°C, and the broth in the dosing and central chambers was agitated by a magnetic stirrer. Ten percent Muller-Hinton broth was used (10).

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▽ Published ahead of print on 5 October 2009.

TABLE 1. AUC_{24}/MIC ratios required for 24-h bacteriostatic effect and 1-, 2-, and 3-log reductions in bacterial count

Strain or analysis	MIC (mg/liter)	AUC_{24}/MIC required for:			
		24-h bacteriostatic effect	1-log drop	2-log drop	3-log drop
Strain					
36633	0.03	53.7	61.7	70.8	83.2
37099	0.03	41.7	49.0	56.2	67.6
37312	0.03	18.6	22.4	26.3	30.9
37390	0.04	12.0	15.1	18.6	25.1
37503	0.05	20.4	30.2	45.7	85.1
38002	0.05	16.6	19.1	21.4	25.1
38004	0.09	8.7	19.5	41.7	95.5
37276	0.7	30.2	43.7	64.6	120.0
36742	1	19.5	20.9	23.4	25.4
36945	2	42.7	52.7	89.1	
Mean ± SD		26.4 ± 15.0	33.4 ± 16.8	45.8 ± 24.0	62.0 ± 36.3
Pooled single-point analysis		18.6	26.9	38.9	69.2

Exposure-response studies. Experiments were performed with an initial inoculum density of 10 CFU/ml (6). A total of 720 μl of a 10-CFU/ml (10) bacterial suspension from a 24-h plate was added to the central chamber 45 min before dosing. Samples were taken throughout a 48-h period for determination of viable counts. Bacteria were quantified using a spiral plater (Don Whitley Spiral Systems, Shipley, West Yorkshire, United Kingdom). Bacteria were recovered onto nutrient agar plates. The minimum level of detection was 100 CFU/ml. Additional aliquots were also stored at −70°C for measurement of moxifloxacin using a modified bioassay method (4). The moxifloxacin regimens were based on healthy volunteer pharmacokinetic data, dosed once per day (terminal half-life, 12 h) (13). Dose-ranging simulations were performed for each strain to achieve AUC_{24}/MIC ratios ranging up to 1,586, which is 7 to 12 experiments per bacterial strain. The AUC_{24}/MIC ratios simulated were as follows: AUC/MIC ≤ 1.0 (n = 10), 1.0 ≤ AUC/MIC < 10 (n = 10), 10 ≤ AUC/MIC < 20 (n = 8), 20 ≤ AUC/MIC < 30 (n = 6), 30 ≤ AUC/MIC < 50 (n = 9), 50 ≤ AUC/MIC < 100 (n = 10), 100 ≤ AUC/MIC < 200 (n = 10), and 200 ≤ AUC/MIC (n = 14).

Modeling methods. The antibacterial effect, as measured by the log reduction in viable bacteria count at 24 h, was related to the antibiotic exposure as measured by AUC_{24}/MIC using a Sigmoid Emax model (GraphPad Prism; GraphPad Software Incorporated, San Diego, CA), and the AUC_{24}/MIC required for 24-h bacteriostatic effect and 1-, 2-, and 3-log drops was established for each strain. A similar analysis was performed combining all the data from all 10 strains (pooled analysis) to derive a single AUC_{24}/MIC for each measurement of antibacterial effect.

The percentage of patients reaching each pharmacodynamic target (for 24-h bacteriostatic effect and 1-, 2-, and 3-log drops) was estimated by simulation for a range of pathogen MICs using Stata version 10 (StataCorp LP, College Station, TX). In each run, 10,000 patients were simulated. An AUC was simulated for each patient by first randomly allocating an AUC range of 286 subjects receiving 400 mg oral moxifloxacin, according to the distribution described in Stass and Proeve's study (14), and then drawing the AUC randomly from a uniform distribution within that range.

The distribution described by Stass and Proeve (14) was as follows: AUC ≤ 15 mg · h/liter (n = 0), 15 ≤ AUC < 20 mg · h/liter (n = 4), 20 ≤ AUC < 25 mg · h/liter (n = 18), 25 ≤ AUC < 30 mg · h/liter (n = 58), 30 ≤ AUC < 35 mg · h/liter (n = 62), 35 ≤ AUC < 40 mg · h/liter (n = 65), 40 ≤ AUC < 45 mg · h/liter (n = 34), 45 ≤ AUC < 50 mg · h/liter (n = 23), 50 ≤ AUC < 55 mg · h/liter (n = 15), 55 ≤ AUC < 60 mg · h/liter (n = 3), 60 ≤ AUC < 65 mg · h/liter (n = 2), 65 ≤ AUC ≤ 70 mg · h/liter (n = 1), 70 ≤ AUC < 75 mg · h/liter (n = 0), and 75 ≤ AUC < 80 mg · h/liter (n = 1).

AUC_{24}/MIC targets (free drug) for 24-h bacteriostatic effect and 1-, 2-, and 3-log drops in CFU/ml were simulated for each patient in the following three ways: (i) empirically, using the results for the 10 strains tested, with each patient having an equal probability of being allocated the AUC_{24}/MIC for each of the 10 strains; (ii) assuming a normal distribution of AUC_{24}/MICs, with the means and standard deviations estimated from the 10 test strains; and (iii) using the AUC_{24}/MIC target calculated from the data from all 10 strains pooled, i.e., a single-point analysis. Protein binding was assumed to be 40%, so free-drug AUC was 60% of total simulated AUC. Therefore, a patient was calculated to have attained the AUC_{24}/MIC target if AUC × 0.6/MIC > target. The calculation was repeated for each MIC of 0.06 to 4 mg/liter. The percentage of patients attaining the target was determined for each combination of target calculation method, the log change in count of viable bacteria required, and the pathogen MIC (mg/liter). Thus, the percentage of patients reaching the pharmacokinetic-pharmacodynamic target for each endpoint (24-h bacteriostatic effect and 1-, 2-, and 3-log drops) and pathogen MIC was assessed in three different ways.

RESULTS

MICs for moxifloxacin. The moxifloxacin MICs for the 10 *S. aureus* strains used were as follows: for strain SMH 36633, a MIC of 0.03 mg/liter; SMH 37099, a MIC of 0.03 mg/liter; SMH 37312, a MIC of 0.03 mg/liter; SMH 37390, a MIC of 0.04 mg/liter; SMH 37503, a MIC of 0.05 mg/liter; SMH 38002, a MIC of 0.05 mg/liter; SMH 38004, a MIC of 0.09 mg/liter; SMH 37276, a MIC of 0.7 mg/liter; SMD 36742, a MIC of 1.0 mg/liter; and SMD 36945, a MIC of 2.0 mg/liter.

Impact of escalating exposures of moxifloxacin on total bacterial populations. The free-drug moxifloxacin AUC_{24}/MIC required to produce a bacteriostatic effect at 24 h and 1-, 2-, and 3-log reductions in bacterial count for each strain are shown in Table 1, together with the mean ± the standard deviation for the 10 strains and the single-point AUC_{24}/MICs from the pooled analysis. There was variation between strains in the AUC_{24}/MIC ratio to produce a similar log reduction in count of viable bacteria at 24 h. The mean AUC_{24}/MICs of the 10 individual strains were similar to the estimates from the pooled data single-point analysis.

Achieving the targeted AUC_{24}/MICs. The percentage of simulated patients achieving the target AUC_{24}/MIC ratio for each pathogen MIC is shown in Table 2. The empirical and normal-based methods of accounting for variation in the AUC_{24}/MIC target gave generally similar results, although the distribution of AUC_{24}/MIC targets for the 10 strains was skewed to the right (that is, having a tail of strains with high AUC_{24}/MIC targets). Use of a single pooled estimate of the AUC_{24}/MIC target, compared with estimates accounting for variation in the target, gave higher target attainment rates for lower MICs and lower rates for higher MICs. Assuming the sus-

TABLE 2. Percentage of patients achieving target AUC_{24}/MICs

Estimation	MIC (mg/liter)	% of patients with target AUC_{24}/MICs (log_{10} drop in CFU/ml at 24 h)			
		0	−1	−2	−3
Empirical estimation of the distribution of targets	0.06	100	100	100	89.7
	0.12	100	100	99.9	88.8
	0.25	99.4	98.4	89.4	61.4
	0.5	80.6	68.1	49.5	38.0
	1.0	46.8	31.2	16.4	7.5
	2.0	10.2	0.8	0.2	0
Normal-based estimation of distribution of targets	0.06	100	100	100	100
	0.12	100	100	99.9	98.6
	0.25	99.4	98.3	89.6	71.6
	0.5	81.6	68.5	45.5	30.6
	1.0	36.9	25.3	16.0	13.8
	2.0	15.0	9.1	7.3	8.2
Single-point estimation of target using pooled data for all strains	0.06	100	100	100	100
	0.12	100	100	100	100
	0.25	100	100	99.6	77.1
	0.5	99.8	95.5	61.8	1.8
	1.0	68.0	16.0	0.8	0
	2.0	1.1	0	0	0

ceptibility breakpoint for moxifloxacin is the highest MIC for which the target attainment rate is >90% using a bacteriostatic effect target (3), then the clinical breakpoint is based on the following factors: (i) the empirical estimation of the target distribution is S (sensitive) ≤ 0.25 mg/liter; (ii) the normal-based estimation of the target distribution is S ≤ 0.25 mg/liter; and (iii) the single-point estimate of the target is S ≤ 0.5 mg/liter.

DISCUSSION

At present, variation in pathogen MICs, variation in the pharmacodynamic index size related to cross-species differences, and variation in human drug pharmacokinetics are included in the mathematical modeling, often Monte Carlo analysis, when establishing antibiotic dosing regimens and clinical susceptibility breakpoints (8). However, until now, a single pharmacodynamic index magnitude has been used as the pharmacodynamic target, for example, with moxifloxacin and anaerobes (12). It has been recognized that variation in the pharmacodynamic target could be important, and this was embedded in the process of setting clinical breakpoints for daptomycin against *S. aureus* in Europe. In this instance, as well as in establishing a mean AUC/MIC target, worse case (minus standard deviation) and best case (plus standard deviation) analyses were performed (7).

In the analysis performed here, for the first time, we included variation in the pharmacodynamic index (AUC_{24}/MIC) in the mathematical modeling performed to suggest a possible clinical breakpoint for moxifloxacin against *S. aureus*. This was compared to a single-point AUC_{24}/MIC target analysis, such as that usually performed. The single-point analysis of the pooled experimental data from all 10 *S. aureus* strains indicated a clinical breakpoint for susceptibility of ≤0.5 mg/liter; this is the present European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint in Europe and one dilution lower than the CLSI moxifloxacin clinical breakpoint for *S. aureus* (S ≤ 1 mg/liter) (5). Introduction of variation in the pharmacodynamic index target, either based on the right-skewed distribution observed in the 10 strains or a parametric (normal) distribution based on the means and standard deviations from the 10 tested strains, resulted in a lower clinical susceptibility breakpoint (S ≤ 0.25 mg/liter). Although the use of an actual AUC_{24}/MIC target distribution is preferable, the parametric approach produced similar results. However, a log-normal distribution might have advantages compared to a normal one, as it is right skewed. Following either approach, incorporating AUC_{24}/MIC target variability into the mathematical models resulted in the lowering of high target attainment rates and an increase in low attainment rates, as would be expected.

In conclusion, mathematical modeling of the effects of strain-to-strain within-species and experimental variability in the relationship between antibacterial effect and pharmacodynamic index has an impact on determining likely susceptibility breakpoints. Such variability should be incorporated into future mathematical modeling concerning antibiotic dosing, potential target pathogens, and clinical susceptibility breakpoints.

ACKNOWLEDGMENT

This work was financially supported by North Bristol NHS Trust Charitable Funds, fund number 95105.

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