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Use of Pharmacodynamic Parameters To Predict Efficacy of Combination Therapy by Using Fractional Inhibitory Concentration Kinetics

JAN G. DEN HOLLANDER,[1,2]* JOHAN W. MOUTON,[1] AND HENRI A. VERBRUGH[1]

Department of Medical Microbiology and Infectious Diseases, University Hospital Rotterdam Dijkzigt,[1] and Department of Internal Medicine, Zuiderziekenhuis,[2] Rotterdam, The Netherlands

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Combination therapy with antimicrobial agents can be used against bacteria that have reduced susceptibilities to single agents. We studied various tobramycin and ceftazidime dosing regimens against four resistant *Pseudomonas aeruginosa* strains in an in vitro pharmacokinetic model to determine the usability of combination therapy for the treatment of infections due to resistant bacterial strains. For the selection of an optimal dosing regimen it is necessary to determine which pharmacodynamic parameter best predicts efficacy during combination therapy and to find a simple method for susceptibility testing. An easy-to-use, previously described E-test method was evaluated as a test for susceptibility to combination therapy. That test resulted in a MIC_{combi}, which is the MIC of, for example, tobramycin in the presence of ceftazidime. By dividing the tobramycin and ceftazidime concentration by the MIC_{combi} at each time point during the dosing interval, fractional inhibitory concentration (FIC) curves were constructed, and from these curves new pharmacodynamic parameters for combination therapy were calculated (i.e., AUC_{combi}, $C_{max\text{-}combi}$, $T_{>MIC\text{-}combi}$, and $T_{>FICi}$, where AUC_{combi}, $C_{max\text{-}combi}$, $T_{>MIC\text{-}combi}$, and $T_{>FICi}$ are the area under the FIC_{combi} curve, the peak concentration of FIC_{combi}, the time that the concentration of the combination is above the MIC_{combi}, and the time above the FIC index, respectively). By stepwise multilinear regression analysis, the pharmacodynamic parameter $T_{>FICi}$ proved to be the best predictor of therapeutic efficacy during combination therapy with tobramycin and ceftazidime ($R^2 = 0.6821$; $P < 0.01$). We conclude that for combination therapy with tobramycin and ceftazidime the $T_{>FICi}$ is the parameter best predictive of efficacy and that the E-test for susceptibility testing of combination therapy gives promising results. These new pharmacodynamic parameters for combination therapy promise to provide better insight into the rationale behind combination therapy.

In the last decade three important pharmacodynamic parameters which correlate well with therapeutic efficacy in in vitro as well as in animal models have been described. These parameters differentiate between groups of antimicrobial agents with diverse mechanisms of action. For instance, the efficacies of β-lactam antibiotics and erythromycin correlate best with the time that the levels in serum exceed the MIC ($T_{>MIC}$), while for aminoglycosides the area under the concentration-time curve (AUC) best predicts therapeutic efficacy (32). Furthermore, aminoglycosides display concentration-dependent killing in vitro (9, 31) and in vivo (16), indicating the importance of the third pharmacokinetic parameter, i.e., the peak concentration (C_{max}). On the basis of these observations new dosing regimens for these antimicrobial agents are now being used, including aminoglycoside dosing regimens that were changed from thrice daily to once daily (22, 28).

However, all these pharmacodynamic studies used single agents and the pharmacodynamic parameters for combination therapy are still lacking. Parameters which have been used to show interactions during combination therapy are the fractional inhibitory concentration (FIC) indices (FICis), derived from checkerboard titrations (2, 3, 6, 11, 14, 15, 24). Alternatively, a significant change in the killing rates observed in time-kill experiments has been used (7, 13, 27). Recently, a computer model, the MacSynergy program, has been used to indicate synergism (8). This method provides us with a rating of synergism expressed as the maximum effect of the drug combination. Although this method is much more accurate in predicting the synergistic effect of two drugs, it does not indicate the pharmacodynamic parameters which predict efficacy.

Unfortunately, the results of the various studies are discordant with the results of checkerboard titrations, the results of time-kill experiments, and clinical outcome (5, 23, 24). In spite of the numerous studies evaluating combination therapy, no pharmacodynamic parameters that can accurately predict the therapeutic efficacy of combination therapy have been found. One of the most important reasons is that all methods described above were based on efficacy at static drug concentrations, while in vivo the concentrations decline over time.

The purpose of the present study was to search for a pharmacodynamic parameter that may predict the therapeutic efficacy of combination therapy. For this purpose, several tobramycin and ceftazidime dosing regimens were simulated in an vitro pharmacokinetic model to study their effect on resistant *Pseudomonas aeruginosa* strains. A simplified version of the checkerboard titration, i.e., an E-test for combination therapy (33), was also included.

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MATERIALS AND METHODS

Theoretical approach. To obtain pharmacodynamic parameters for combination therapy that are comparable to the AUC, C_{max}, and $T_{>MIC}$ for monotherapy, it is not possible to simply add the values of the pharmacodynamic parameters for the different antibiotics. We therefore introduce here new phar-

* Corresponding author. Present address: Department of Internal Medicine, Zuiderziekenhuis, Groene Hilledijk 315, 3075 EA Rotterdam, The Netherlands. Phone: 31-10-2903000, ext. 109. Fax: 31-10-2903361.

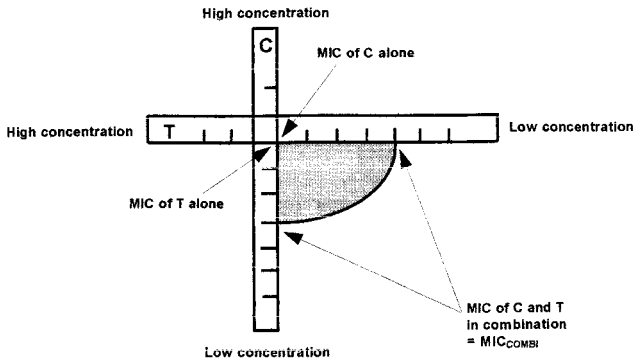

FIG. 1. Schematic diagram of the E-test combination therapy susceptibility test described by White et al. (33).

macodynamic parameters for combination therapy, i.e., the AUC_{combi}, $C_{max\text{-}combi}$, $T_{>MIC\text{-}combi}$, and $T_{>FICi}$ (the area under the FIC_{combi} curve, the peak concentration of FIC_{combi}, the time that the concentration of the combination is above the MIC_{combi}, and the time above the FICi, respectively), which are based on FIC curves and which are explained below. These curves were calculated as follows. The FIC used in the checkerboard titration to calculate FICi is defined as the concentration of antibiotic Y1 (in the presence of drug Y2) in a well (C_W) divided by the MIC of that drug for the strain (2, 3, 10) and is expressed as $FIC_Y = C_W/MIC$ (equation 1). The FICi is then calculated as $\Sigma(FIC_{Y1} + FIC_{Y2})/n$ (equation 2), where Y1 and Y2 are the two antibiotics, respectively, and n is the number of wells used to calculate the sum of the FICs.

However, in checkerboard titrations the concentrations of the antibiotics are constant in each well. During the dosing regimens in the in vitro model these concentrations change over time during the dosing interval. Therefore, we simulated the concentration-time curves for the individual drugs. At time intervals of 0.1 h a FIC at time t (FIC_t) was calculated for the concentration at that time (C_t) by equation 1 and was comparable to the checkerboard titration as $FIC_t = C_t/MIC$ (equation 3), resulting in a FIC curve over time.

However, equation 3 uses the MIC during exposure to a single agent. During combination therapy it is likely that the MIC of each drug changes in the presence of the other drug; i.e., the MIC decreases if the drugs are acting synergistically. A recently described method (33) for susceptibility testing during combination therapy is based on the E-test and has provided us with a new method of determining the MIC of tobramycin in the presence of ceftazidime (and vice versa), which is further called the MIC_{combi} and which could be used as a parameter for describing the susceptibility of a strain during combination therapy. To obtain FIC curves for combination therapy, the concentrations at each time point were divided by the MIC_{combi} rather than the MIC, and these are expressed as: $FIC_{t,combi} = C_t/MIC_{combi}$ (equation 4). The FIC_{combi} curve was then calculated by adding the FIC of tobramycin and the FIC of ceftazidime at the same time point in the concentration-time curves, expressed as $FIC_{combi} = FIC_{t,\,tobra} + FIC_{t,\,cefta}$ (equation 5), where $FIC_{t,\,tobra}$ and $FIC_{t,\,cefa}$ are FIC_ts for tobramycin and ceftazidime, respectively, resulting in FIC_{combi} curves against time. From these FIC_{combi} curves the new pharmacodynamic parameters AUC_{combi}, $C_{max\text{-}combi}$, and $T_{>FICi}$ were calculated. The $T_{>MIC\text{-}combi}$ is the time that the concentration of one or both of the antibiotics is above the MIC_{combi}. The time above the MIC_{combi} ($T_{>MIC\text{-}combi}$) was calculated from the concentration-time curves for the different antibiotics by the same method normally used to calculate the time above the MIC, but for $T_{>MIC\text{-}combi}$ the MIC_{combi} was used.

Bacterial strains, antibiotics, and media. Four nonmucoid *Pseudomonas aeruginosa* strains which were isolated from the sputa of cystic fibrosis patients (CF 133, CF 5706, CF 5846, and CF 5879, respectively) were used in this study. The MICs of tobramycin (Eli Lilly & Company, Nieuwegein, The Netherlands) and ceftazidime (Glaxo, Zeist, The Netherlands) were determined by a standard macrodilution method (21) in Mueller-Hinton broth (Difco, Amsterdam, The Netherlands) supplemented with Ca^{2+} (25 mg/liter) and Mg^{2+} (12.5 mg/liter) (MHBs), as well as by the E-test technique (AB-Biodisk, Solna, Sweden) with Mueller-Hinton agar (Difco) supplemented with Ca^{2+} (25 mg/liter) and Mg^{2+} (12.5 mg/liter). All strains were resistant or intermediately susceptible to both tobramycin and ceftazidime. All samples used for determination of CFU counts were plated onto Trypticase soy agar (Oxoid, Basingstoke, Hampshire, England). The mechanism of resistance for aminoglycosides was determined as described by Van de Klundert et al. (29) by identification of the aminoglycoside-modifying enzymes involved. The mechanism of resistance for β-lactam antibiotics was determined by semiquantitative susceptibility testing, substrate analysis, and isoelectric focusing of the extracted β-lactamase (30).

FICis. FICis were determined both by a modified macrodilution checkerboard macrotitration technique (14) and by an E-test technique (33) (Fig. 1). The FICs and FICis were calculated as usual (2, 10). Synergism by the modified macrodilution checkerboard technique was defined as a FICi of ≤0.8 and indifference was defined as a FICi of between 0.8 and 4.0 (15). For the E-test method synergism was defined as a FICi of ≤0.5 and indifference was defined as a FICi of between ≥0.5 and ≤4.0, comparable to the definitions used for twofold dilution checkerboard titrations (27).

In vitro pharmacokinetic model. The pharmacokinetic model used in this study was previously described in detail (20). Briefly, a two-compartment model consisting of one central compartment and four peripheral compartments (disposable dialyzer units, model ST23; Baxter, Utrecht, The Netherlands) was used to expose the bacteria in the peripheral compartments to changing antibiotic concentrations that mimic the pharmacokinetics in humans. At time zero the peripheral compartments were inoculated with a logarithmic-phase culture of *P. aeruginosa* of approximately 5×10^5 CFU/ml, with a different strain used in each peripheral compartment. Control growth in the model was determined in the same way but without the addition of antibiotics.

Dosing regimens. Fourteen different dosing regimens were applied, with peak concentrations of 32, 16, 8, and 4 mg/liter for tobramycin and 128, 64, and 32 mg/liter for ceftazidime. The drugs were given simultaneously (i.e., tobramycin at time zero followed by ceftazidime at 20 min, or vice versa) or nonsimultaneously (i.e., tobramycin at time zero and ceftazidime at 4 h, or vice versa). During the simultaneous dosing regimens tobramycin was given thrice daily or once daily. The half-lives of both tobramycin and ceftazidime was adjusted to 2 h. Samples were taken at 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 9, 12, 16, and 24 h. The samples were immediately washed (twice) with cold phosphate-buffered saline, and 0.1-ml samples were plated onto Trypticase soy agar plates (limit of detection, 10 CFU/ml). Samples were assayed for tobramycin by a fluorescence polarization immunoassay with a TDxFLx instrument (Abbott Diagnostic Division, Amstelveen, The Netherlands) and for ceftazidime by high-performance liquid chromatography, as described earlier (19). The lower limits of sensitivity of both assays were 0.5 mg/liter. The between-day, between-sample variation was less than 7%.

Data analysis. The pharmacodynamic parameters AUC, C_{max}, and $T_{>MIC}$ for the individual drugs were calculated from simulated concentration-time curves by the equation for an open-compartment model after extravascular administration (25). The area under the killing curve from time zero to 24 h ($AUKC_{0-24}$) was calculated by using the trapezoidal rule on logarithmically transformed, experimentally obtained datum points.

Statistical analysis. The peak and trough concentrations and half-lives of the antibiotics during the different experiments were compared by using a two-way analysis of variance and Tukey's test for multiple comparison of significance with the Instat 2 computer package (11). A P value of ≤0.05 (two tailed) was considered significant.

The correlation between the four pharmacodynamic parameters (AUC_{combi}, $C_{max\text{-}combi}$, $T_{>MIC\text{-}combi}$, and $T_{>FICi}$) and efficacy (i.e., change in CFU per milliliter = $\log_{10}$ CFU per milliliter at 24 h − $\log_{10}$ CFU per milliliter at time zero or $AUKC_{0-24}$) were calculated by stepwise multilinear regression analysis with the SAS computer package (26). The F test was used to choose the best model.

RESULTS

MICs and FICs. The MICs and the MIC_{combi}s of tobramycin and ceftazidime for the four strains were determined by the E-test method and are presented in Table 1. Also presented in Table 1 are the FICis determined by the E-test and a modified macrodilution checkerboard titration method. The MICs determined by a macrodilution standard assay, were not significantly different from those determined by the E-test (data not shown). The calculated values of the FICis obtained by using the MICs obtained by the macrodilution assay differed some-

TABLE 1. Analysis of susceptibility tests showing synergy between tobramycin and ceftazidime against four *P. aeruginosa* strains

Strain	MIC (mg/liter)[a]		MIC_{combi} (mg/liter)[b]		FICi[c]	
	Tobramycin	Ceftazidime	Tobramycin	Ceftazidime	E-test	Macrodilution method
CF 133	32	64	6	16	0.44	0.37
CF 5706	128	16	32	6	0.63	0.68
CF 5846	12	512	6	128	0.75	0.67
CF 5879	16	512	1.5	8	0.10	0.39

[a] Determined by E-test.
[b] Determined by combination E-test.
[c] Calculated; see Materials and Methods.

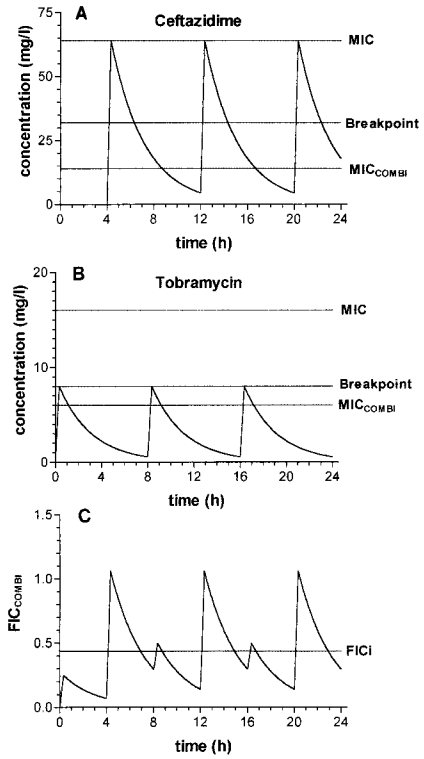

FIG. 2. Representative concentration-versus-time curves for ceftazidime (A) and tobramycin (B) and the corresponding FIC_{combi}-versus-time curve (C) during one combination therapy regimen. In this case the FIC_{combi} curve, the MIC, and the MIC_{combi} are based on data for *P. aeruginosa* CF 133. Breakpoints are according to NCCLS guidelines (19).

what from those obtained by using the MICs obtained by the E-test, but for all four strains the two calculations resulted in the same conclusion, i.e., that there is synergism or indifference.

Mechanism of resistance. All four strains produced a β-lactamase which was identified as a stably depressed, chromosomally encoded class I β-lactamase (4). The mechanism of resistance for tobramycin was due to the production of several aminoglycoside modifying enzymes, which were identified as AAC(6′)-II and APH(3′) for strains CF 133 and CF 5706, APH(3′) for strain CF 5846, and ANT(2″) and APH(3′) for strain CF 5879.

Pharmacokinetics and pharmacodynamics of combination therapy. The peak and trough concentrations and the half-lives did not differ significantly between the experiments and were comparable to the values targeted for these experiments. On the basis of these data, the concentration-time curves and the FIC_{combi} curves were simulated. An example of a simulation of the concentration-versus-time curves for tobramycin and ceftazidime during a nonsimultaneous dosing regimen and the calculated FIC_{combi} curve for this particular regimen are presented in Fig. 2. This combination therapy regimen results in FIC_{combi} curves with values that cycle between 0.1 and 1.1 from 0 to 24 h.

The correlation between the values of the pharmacodynamic parameters (AUC_{combi}, $C_{max\text{-}combi}$, $T_{>MIC\text{-}combi}$, and $T_{>MIC\text{-}combi}$) for all dosing regimens and the Δlog_{10} CFU per milliliter are presented in Fig. 3. The $T_{>FICi}$ and the $T_{>MIC\text{-}combi}$ showed a linear relation with efficacy, and AUC_{combi} and $C_{max\text{-}combi}$ showed a log-linear relation with efficacy. The correlation between the four pharmacodynamic parameters and the $AUKC_{0-24}$ was less than that between the four parameters and the Δlog_{10} CFU per milliliter over 24 h but showed the same trend for the importance of the parameters (Table 2). The most important parameter predicting ef-

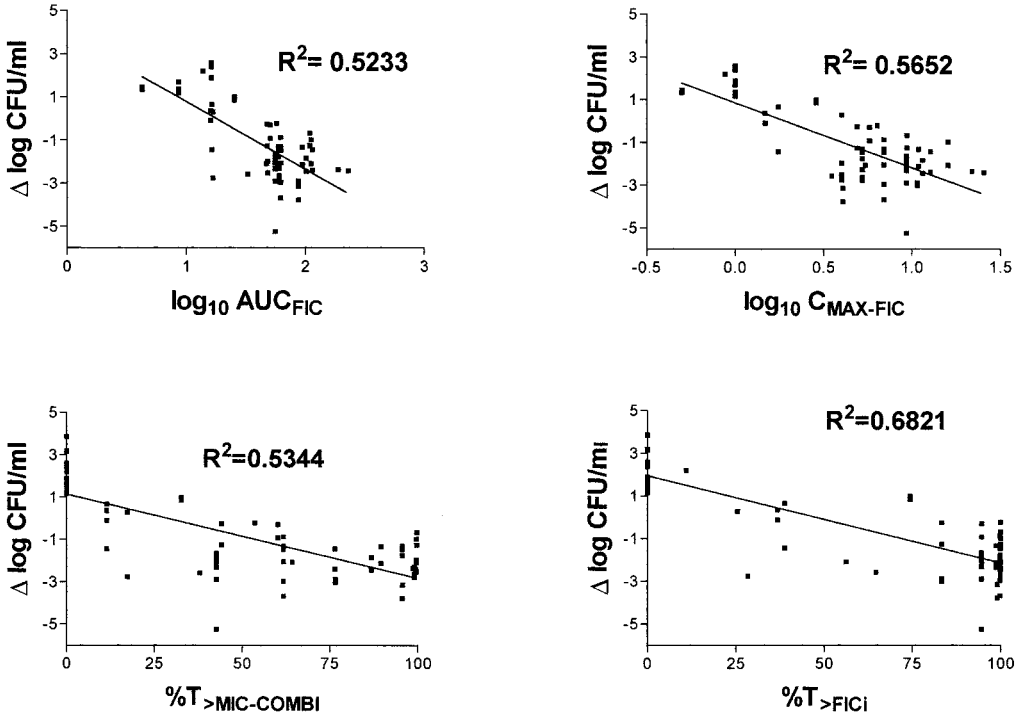

FIG. 3. Correlation between pharmacodynamic parameters for combination therapy and efficacy, expressed as Δlog_{10}CFU per milliliter.

TABLE 2. Correlation between pharmacodynamic parameters and efficacy during combination therapy

Pharmacodynamic parameter	Range of values tested	R^2	
		$\Delta\log_{10}$ CFU/ml at 24 h	$AUKC_{0-24}$[a]
AUC_{combi} (h)	0–230	0.5233	0.3070
$C_{max\text{-}combi}$	0–25.5	0.5652	0.3929
$T_{>MIC\text{-}combi}$ (h)	0–23.9	0.5344	0.3520
$T_{>FICi}$ (h)	0–24	0.6821	0.5350

[a] $AUKC_{0-24}$ calculated as $\log_{10}$ CFU · h/ml.

ficacy was $T_{>FICi}$, as shown by the coefficient of determination (R^2) for all four strains and all regimens together, which was 0.6821. For strain CF 133 enough data were available for the calculation of R^2 for the individual parameters. The R^2 values for this strain showed the same trend as the R^2 values for the four strains but were higher. For the most important parameter, $T_{>FICi}$, R^2 was 0.7604 (data not shown in Table 2).

DISCUSSION

We studied various dosing regimens for combination therapy to determine whether combination therapy may be efficacious against resistant strains and, if so, to determine what pharmacodynamic parameter(s) may best predict efficacy. Recently, we showed that therapy with a combination of tobramycin and ceftazidime was effective against a *P. aeruginosa* strain resistant to both drugs (7). The use of combination therapy that has a synergistic or additive effect may thus be a strategy for treating patients with infections due to multiply resistant strains. For the selection of the optimal dosing regimens for combination therapy, two important factors should be known. First, a method which indicates the susceptibility of a bacterial strain during combination therapy is needed, and second, the pharmacodynamic parameter(s) that predicts efficacy should be elucidated. In this study of combination therapy of tobramycin with ceftazidime against resistant *Pseudomonas* strains, both objectives were goals.

Recently, White et al. (33) developed an easy method of calculating the FICi from MIC data obtained with E-test strips. By their method, it is possible to determine the MIC of tobramycin in the presence of ceftazidime and vice versa, thus providing a MIC_{combi} of each drug. If a combination of drugs with synergistic or additive activity is used, a decrease in the MIC of the combination compared to the MIC of the individual drug is seen. To evaluate whether a strain was susceptible to tobramycin during combination therapy, the breakpoints for the individual drugs were used initially. Thus, it was shown that *P. aeruginosa* CF 133, which was resistant to both tobramycin and ceftazidime according to the breakpoints of the National Committee for Clinical Laboratory Standards (NCCLS) (21), appears to be susceptible to both antibiotics if they are used in combination (i.e., the MIC_{combi} was below the NCCLS breakpoint for monotherapy). This observation explains our earlier finding that this strain was killed during an in vitro simulation of the combination therapy regimens commonly used in cystic fibrosis patients suffering from *P. aeruginosa* infections of the lung (7). For all four strains the MIC_{combi}s were lower than the MICs (Table 1), and as was to be expected, all strains were killed in the in vitro pharmacokinetic model if combination therapy with tobramycin and ceftazidime was simulated. Indeed, by the various regimens, all strains were killed to some extent as measured by the $\Delta\log_{10}$ CFU per milliliter at 24 h. Compared to the NCCLS susceptibility breakpoint for tobramycin or ceftazidime (21), the MIC_{combi}s for two strains (strains CF 133 and CF 5879) were below these breakpoints for both antibiotics, while for the other two strains one of either of the two MIC_{combi}s was below the NCCLS breakpoint (Table 1). This may explain why all four strains behaved as if they were susceptible during time-kill experiments in the pharmacokinetic model, since they were susceptible to at least one of the two antibiotics. Thus, it may be concluded that if the MIC_{combi} of at least one of the drugs used during combination therapy is below the NCCLS breakpoint, it is to be expected that the microorganism will be killed during combination therapy. Since no susceptibility breakpoints for combination therapy have been published, the data presented in this report suggest that if the MIC_{combi} is lower than the NCCLS breakpoints (based on monotherapy regimens), the MIC_{combi} is a reasonable predictor of susceptibility during combination therapy. These observations indicate only that, at least for the four strains used, the susceptibility during combination therapy can be predicted by the E-test method (33). However, this is only based on the results for four strains, and it is therefore too preliminary to introduce this test as a new standard for testing susceptibility to combination therapy. It only suggests a new line of research that seems worthy of examination. Further in vitro and in vivo experiments are needed to further confirm this or to develop new breakpoints for combination therapy.

A similar relation between in vitro data and the in vivo susceptibility of a resistant strain was shown by Mordenti et al. (18). They compared data derived from standard in vitro time-kill experiments and similar tests in an animal model combining amikacin with ticarcillin. They showed that the lowest concentration of the drugs that was still synergistic in standard time-kill experiments predicted whether a resistant strain would be susceptible during combination therapy. However, the use of time-kill experiments is far more laborious than the use of the E-test method recently described by White et al. (33).

To study the pharmacodynamic principles of combination therapy in a way similar to that used for monotherapy (31, 32), new parameters are needed. Such new parameters (AUC_{combi}, $C_{max\text{-}combi}$, and $T_{>FICi}$) obtained with the use of FIC curves and a fourth parameter (i.e., $T_{>MIC\text{-}combi}$) that could be estimated from the concentration-time curves were proposed in this report. A stepwise linear regression analysis of these four new pharmacodynamic parameters for combination therapy revealed that $T_{>FICi}$ is the most important parameter that predicts the efficacy ($P < 0.01$) of tobramycin and ceftazidime combinations against *P. aeruginosa*. For all four strains together this parameter showed a reasonable correlation with the $\Delta\log_{10}$ CFU per milliliter at 24 h ($R^2 = 0.6821$); an even better correlation was found for strain CF 133 alone ($R^2 = 0.7604$). The fact that the $T_{>FICi}$ is important may explain why the use of nonsimultaneous dosing regimens will result in greater killing than that from simultaneous dosing of these agents (1, 12, 17), since the nonsimultaneous dosing regimens provide longer $T_{>FICi}$s compared to those provided by the simultaneous dosing regimens. However, due to variability in the data these correlations may seem overinterpreted, but the multilinear regression analysis and the statistical tests show significant correlations. Even though there is variability in the data, the correlations between the four pharmacodynamic parameters and efficacy suggest that all parameters are linked to efficacy, and further research along these lines is needed to reveal the right correlations for all kinds of combination therapy.

In conclusion, we described a simple method of determining the susceptibility of a strain during combination therapy

and propose new pharmacodynamic parameters (AUC_{combi}, $C_{max\text{-}combi}$, $T_{>FICi}$, and $T_{>MIC\text{-}combi}$) which predict the efficacy of the combination therapy; of these, $T_{>FICi}$ seems to be correlated best with the efficacy of combination therapy with tobramycin and ceftazidime. The efficacies of other drug combinations may well be best predicted by other pharmacodynamic parameters. Such knowledge would provide a rationale for dosing regimens with combination therapy and may provide us with optimal dosing regimens for the treatment of patients with infections caused by multiply resistant bacterial strains.

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ADDENDUM

A stepwise description of the dynamic FIC curves is as follows.

(i) Determine the MIC_{combi} for each drug and strain by the method of White et al. (33).

(ii) Calculate the concentration-time profile for the drug regimen, comparable to Fig. 2A and B.

(iii) Divide for each time point the actual drug concentration by the MIC_{combi} of that drug. Add the two FICs at that time point and plot those against time. This results in the dynamic FIC_{combi} profile shown in Fig. 2C.

(iv) Use this FIC_{combi}-versus-time profile to calculate three new pharmacodynamic parameters: AUC_{combi}, $C_{max\text{-}combi}$, and $T_{>FICi}$.

(v) Calculate the $T_{>MIC\text{-}combi}$, which is the time during which at least one of the drug concentrations is above the MIC_{combi}, from the two drug concentration-versus-time profiles (Figure 2A and B).

(vi) The therapeutic effect can be expressed as the Δlog CFU per milliliter at 24 h and as the $AUKC_{0-24}$.

(vii) Try to find a correlation between the pharmacodynamic parameters for combination therapy and the therapeutic effect.

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