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Parameters of Bacterial Killing and Regrowth Kinetics and Antimicrobial Effect Examined in Terms of Area under the Concentration-Time Curve Relationships: Action of Ciprofloxacin against *Escherichia coli* in an In Vitro Dynamic Model

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Although many parameters have been described to quantitate the killing and regrowth of bacteria, substantial shortcomings are inherent in most of them, such as low sensitivity to pharmacokinetic determinants of the antimicrobial effect, an inability to predict a total effect, insufficient robustness, and uncertain interrelations between the parameters that prevent an ultimate determination of the effect. To examine different parameters, the kinetics of killing and regrowth of *Escherichia coli* (MIC, 0.013 μg/ml) were studied in vitro by simulating a series of ciprofloxacin monoexponential pharmacokinetic profiles. Initial ciprofloxacin concentrations varied from 0.02 to 19.2 μg/ml, whereas the half-life of 4 h was the same in all experiments. The following parameters were calculated and estimated: the time to reduce the initial inoculum (N_0) 10-, 100-, and 1,000-fold ($T_{90\%}$, $T_{99\%}$, and $T_{99.9\%}$, respectively), the rate constant of bacterial elimination (k_{elb}), the nadir level (N_{min}) in the viable count (N)-versus-time (t) curve, the time to reach N_{min} (t_{min}), the numbers of bacteria that survived (N_τ) by the end of the observation period (τ), the area under the bacterial killing and regrowth curve (log N_A-t curve) from the zero point (time zero) to τ (AUBC), the area above this curve (AAC), the area between the control growth curve (log N_C-t curve) and the bacterial killing and regrowth curve (log N_A-t curve) from the zero point to τ (ABBC) or to the time point when log N_A reaches the maximal values observed in the log N_C-t curve (I_E; intensity of the effect), and the time shift between the control growth and regrowth curves (T_E; duration of the effect). Being highly sensitive to the AUC, I_E and T_E showed the most regular AUC relationships: the effect expressed by I_E or T_E increased systematically when the AUC or initial concentration of ciprofloxacin rose. Other parameters, especially $T_{90\%}$, $T_{99\%}$, $T_{99.9\%}$, t_{min}, and $\log N_0 - \log N_{min} = \Delta \log N_{min}$, related to the AUC less regularly and were poorly sensitive to the AUC. T_E proved to be the best predictor and t_{min} proved to be the worst predictor of the total antimicrobial effect reflected by I_E. Distinct feedback relationships between the effect determination and the experimental design were demonstrated. It was shown that unjustified shortening of the observation period, i.e., cutting off the log N_A-t curves, may lead to the degeneration of the AUC-response relationships, as expressed by $\log N_0 - \log N_\tau = \Delta \log N_\tau$, AUBC, AAC, or ABBC, to a point where it gives rise to the false idea of an AUC- or concentration-independent effect. Thus, use of I_E and T_E provides the most unbiased, robust, and comprehensive means of determining the antimicrobial effect.

Dynamic models simulating antibiotic pharmacokinetics have been proven to be useful tools in in vitro studies with antimicrobial agents, especially studies with agents with similar antimicrobial activities but different pharmacokinetic patterns (2, 3, 13, 17, 18, 24). However, all too often the true potential of this approach could not be exploited due to the lack of any quantitation of the bacterial killing and regrowth curves or to the use of inappropriate measures of the antimicrobial effect. Most currently relevant parameters are schematically presented in Fig. 1. Some of them, the time to reduce the initial inoculum (N_0) 10-, 100-, and 1,000-fold, i.e., $T_{90\%}$ (30), $T_{99\%}$ (36), and $T_{99.9\%}$ (37), respectively, are usually considered measures of initial killing and are widely used in many studies. However, the observed reduction of viable counts may be treated only as the net result of two competitive processes: continuing growth of a certain portion of the bacterial population and the true killing of another portion (22, 38, 39). From this point of view, a slope of the time-dependent changes in the difference between logarithms of viable counts with antibiotic (N_A) and without antibiotic (N_C; control growth), i.e., the rate constant of elimination of bacteria (k_{elb}; not shown in Fig. 1), may be preferred to $T_{90\%}$, $T_{99\%}$, or $T_{99.9\%}$ (6).

The minimum number of bacteria resulting from exposure to antibiotic (N_{min} [31]) and the time to reach it (t_{min} [31]) are usually considered measures of complete killing, although, in fact, both describe the state of equilibrium between the growth and killing of bacteria and may be referred to as estimates of intermediate killing. Based on these considerations, the term "total killing" was introduced (32), which, for example, may be expressed by the duration of the effect. It was first described as the time interval (T_d) between the zero point (time [t] = 0), when N_A is equal to N_0, and the time to return to the same bacterial numbers in the regrowth phase (25, 27) and was later described as the time shift (T_E) between the normal growth

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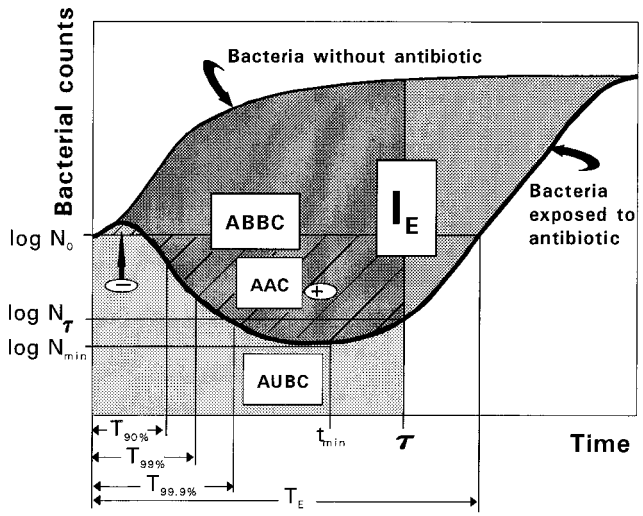

FIG. 1. Parameters for quantitating bacterial killing and regrowth curve and the antimicrobial effect.

and the regrowth curves (14). To express the extent of the reduction of bacterial numbers, another measure describing the number of viable counts (N_τ) at the end of the observation period that usually mimicked the dosing interval (τ) was introduced (34).

The appearance of some integral parameters which simultaneously consider both time and amplitude dimensions of bacterial killing-time curves was a logical development of the idea of combining T_d or T_E with N_τ. The oldest of these integral parameters, AUBC, describes the area under the log N_A-time curve (26, 32). A more recent parameter, AAC, describing the area above the log N_A-t curve and under the baseline drawn at the level of N_A equal to N_0 (33) is, in fact, the algebraic sum of the areas around the N_0 level. Unlike AUBC or AAC, the area between control growth and the bacterial killing and regrowth curves (ABBC) is a direct measure of the antimicrobial effect, since ABBC considers both log N_A-time and log N_C-time curves (15). A similar parameter has been defined as the bacteriolytic area (16).

Generally speaking, all the events occurring from the moment of initial deflection of the time-kill curve from the control growth curve up to the moment of subsequent rapprochement of these curves (at the end of the regrowth phase) may be attributed to the antimicrobial effect. Since AUBC, AAC, and ABBC are determined within an arbitrarily chosen interval (τ), which may or may not incorporate the regrowth phase, these parameters may or may not describe the total effect. To express it completely, another integral parameter, I_E, the area between the control growth and bacterial killing and regrowth curves determined to the end of the regrowth phase, has been described as the intensity of the antimicrobial effect (10–12).

This study was designed (i) to compare the parameters described above in terms of the respective area under the concentration-time curve (AUC)–response relationships as applied to the action of ciprofloxacin against *Escherichia coli*, (ii) to establish interrelationships between different parameters, with special reference to their ability to predict the total effect, and (iii) to examine feedback relationships between quantitation of the data and the experimental design.

MATERIALS AND METHODS

Antibiotic and bacterial strain. Ciprofloxacin lactate and clinical isolate *E. coli* 11775 were used in the study. The MIC determined in Mueller-Hinton broth at the inoculum size of 10^6 CFU/ml was 0.013 μg/ml.

Simulated pharmacokinetic profiles. A series of monoexponential pharmacokinetic profiles of ciprofloxacin with an elimination half-life ($t_{1/2}$) of 4 h were simulated in vitro. The respective initial concentrations (C_0) ranged from 0.019 to 19.2 μg/ml by using twofold steps. The respective values of the AUC from the zero point, i.e., the moment of antibiotic administration into the dynamic model, to infinity were calculated analytically by using pharmacokinetic parameters of ciprofloxacin in humans (19, 35).

In vitro dynamic model and operating procedure. A simplified version of the in vitro dynamic model described previously (14, 30) was used in the study. Briefly, the model consisted of two connected flasks, one of them containing fresh Mueller-Hinton broth and the other, the central unit, with the same broth containing only a bacterial culture (control growth experiments) or bacterial culture plus antibiotic (killing and regrowth experiments). The central unit was incubated at 37°C in a shaking water bath. Peristaltic pumps (Minipuls 2; Gilson) circulated fresh nutrient medium to the bacteria or medium containing the bacteria and antibiotic and from the central 40-ml unit at a flow rate of 7 ml/h. Hence, the clearance provided by the chosen flow rate together with the volume of the central unit ensured monoexponential elimination of ciprofloxacin and bacteria from the system at an elimination rate constant of 0.17 h^{-1} ($t_{1/2}$ = 4 h). Accurate simulations of the desired pharmacokinetic profiles were provided by maintaining a constant volume of the central unit and a constant flow rate, as also proved by direct high-pressure liquid chromatography determinations (data not shown).

The system was filled with sterile Mueller-Hinton broth and placed in an incubator thermostated at 37°C. The central unit was inoculated with an 18-h culture of *E. coli*. After a 2-h incubation of the bacteria, ciprofloxacin was injected into the central unit, and the resulting exponentially growing cultures amounted to approximately 10^6 CFU/ml. In each case, the duration of the experiments was defined by the time for the bacteria that survived to reach the maximum numbers observed without antibiotic. In any event, the experiments were stopped when log N_A reached at least 10^{11} CFU/ml.

Quantitation of bacterial growth and killing. In each experiment, 0.1-ml samples were withdrawn from the central unit, at first every 30 min, later hourly, then every 3 h, and then again hourly, throughout the observation period. These samples were subjected to serial 10-fold dilutions with chilled, sterile 0.9% NaCl and were plated in duplicate on Mueller-Hinton agar. After incubation at 37°C the resulting bacterial colonies were counted and the numbers of CFU per milliliter were calculated. Incubation times varied from 16 to 24 h.

Quantitative evaluation of the antimicrobial effect. For each curve, if applicable, $T_{90\%}$, $T_{99\%}$, $T_{99.9\%}$, k_{elb}, N_{min} (as the difference log N_0 − log N_{min} = Δ *log* N_{min}), t_{min}, N_τ (as the difference log N_0 − log N_τ = Δ log N_τ), T_E, AUBC, AAC, ABBC, and I_E were estimated or calculated. To determine k_{elb}, the log N_A-time data sets reflecting the initial decrease in N_A were converted into the respective differences between log N_A and log N_0.

To examine the robustness of T_E and I_E, their values were estimated and calculated at various levels on the normal growth and antibiotic-induced killing

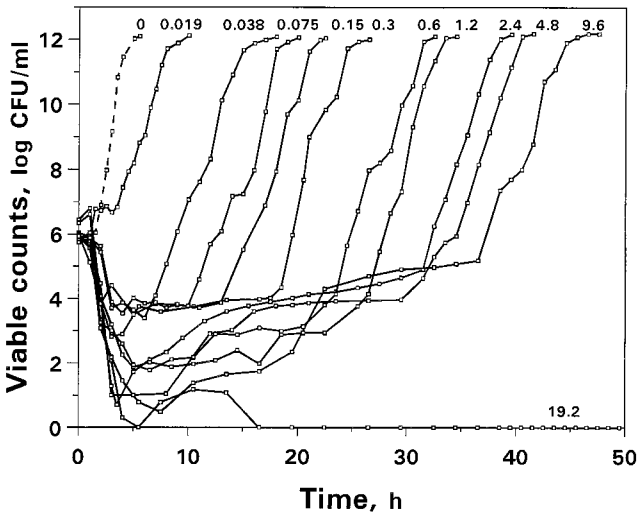

FIG. 2. Killing and regrowth kinetics of *E. coli* exposed to exponentially decreasing concentrations of ciprofloxacin. The number above each curve denotes the respective initial concentration of ciprofloxacin (in micrograms per milliliter).

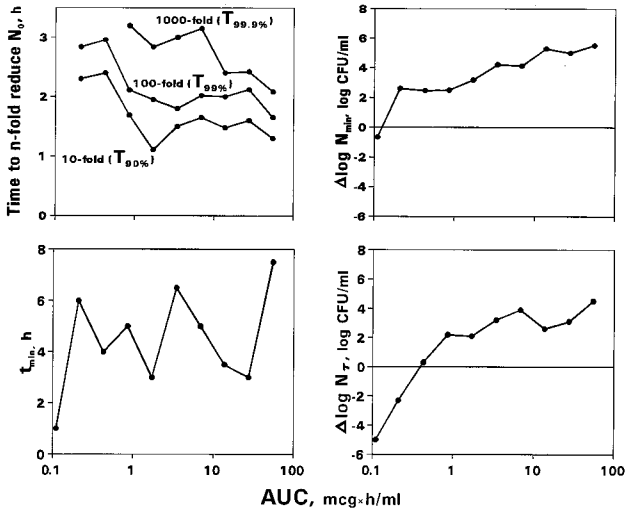

FIG. 3. AUC-dependent changes in the point parameters of bacterial killing and regrowth curves.

and regrowth curves at 10^6, 10^8, and 10^{10} and at 10^8, 10^{10}, and 10^{11} CFU/ml, respectively. To examine the possible impact of the experimental design on Δ log N_τ, AUBC, AAC, and ABBC, the values of these parameters were determined at various durations of the observation period, at τ equal to 12 h (usual dosing interval for ciprofloxacin) as well as at τ equal to 6 and 24 h.

Correlation and regression analyses of the relationships between each of the parameters and the AUC as well as between different parameters and different estimates of a given parameter were performed by using STATISTICA software (version 4.3; StatSoft, Inc.).

RESULTS

The killing and regrowth kinetics of *E. coli* exposed to exponentially decreasing concentrations of ciprofloxacin are presented in Fig. 2. As seen in Fig. 2, at C_0s ranging from 0.038 to 9.6 μg/ml, a distinct bacterial regrowth was followed by a pronounced reduction in viable counts. At the lowest C_0 tested (0.019 μg/ml) the bacterial numbers did not fall at all, although the time course of log N_A differed from that of log N_C (C_0 = 0), whereas at the highest C_0 tested (19.2 μg/ml) no regrowth occurred. The pattern of bacterial regrowth observed at C_0 ranging from 0.038 to 9.6 μg/ml was similar. An increase in C_0 was accompanied by a systematic shifting of the regrowth portions of the log N_A-time curves over time, while the initial reductions in viable counts were less regular.

AUC-dependent changes in the parameters describing bacterial killing and regrowth curves and the antimicrobial effect. The irregular pattern in the reduction of viable counts mentioned above was confirmed by comparing the $T_{90\%}$, $T_{99\%}$, and $T_{99.9\%}$ with the respective AUCs (Fig. 3). As seen in Fig. 3, the $T_{90\%}$-, $T_{99\%}$-, or $T_{99.9\%}$-log AUC curves were erratic, and no regular shortening of the $T_{90\%}$, $T_{99\%}$, and $T_{99.9\%}$ associated with an increase in the AUC could be detected. Moreover, almost 1,000-fold differences in the simulated AUC induced ≤2.2-fold differences in $T_{90\%}$, $T_{99\%}$, and $T_{99.9\%}$. To obtain a more accurate measure of initial bacterial killing, k_{elb} and log N_A − log N_C were plotted versus time (Fig. 4). Because of the curvilinear pattern of these plots, only quasilinear portions were used for determination of k_{elb}. As seen in Fig. 4, the AUC-dependent changes in k_{elb} were more regular, although this parameter was still poorly sensitive to AUC: approximately 2.3-fold differences in k_{elb} corresponded to the large AUC range mentioned above.

t_{min} showed quite irregular AUC dependence (Fig. 3). Unlike t_{min}, the minimum bacterial numbers expressed by Δ log N_{min} were reasonably dependent on the AUC. In general, the Δ log N_{min} values increased systematically with an increase in the AUC and varied within a sixfold range. Another measure of bacterial killing or the bacterial killing and regrowth curve, N_τ, appeared to be even more sensitive to the AUC: a 10-fold range of Δ log N_τ corresponded to a 450-fold range of the simulated AUC (Fig. 3). However, the AUC-dependent increase in Δ log N_τ was less regular compared to that of Δ log N_{min}.

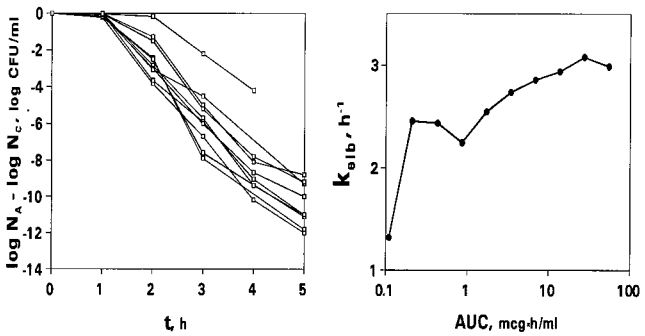

FIG. 4. Time course of log N_A − log N_C differences and AUC-dependent changes in the rate constant of bacteria elimination.

A more regular pattern of the AUC-response curves was provided by integral measures of bacterial killing or the killing and regrowth curve (AUBC) and the antimicrobial effect as such (ABBC). No distortion of the expected AUC-related decrease in AUBC or increase in ABBC was found (Fig. 5), whereas the AUC-dependent increase in AAC was less regular. All three parameters showed a distinct saturation of the effect: there were apparently AUC-independent portions in the curves at high AUCs. However, no saturation was observed in the I_E-log AUC (Fig. 5) and T_E-log AUC (Fig. 6) plots, which were the most regular. Moreover, T_E and, especially, I_E were more sensitive to the AUC than AUBC, AAC, and ABBC. Approximately 20- and 30-fold differences in T_E and I_E, respectively, corresponded to eightfold differences in the AUBC, AAC, or ABBC.

Interrelationships between parameters. Some of the examined parameters, $T_{90\%}$, $T_{99\%}$, $T_{99.9\%}$, t_{min}, Δ log N_{min}, and Δ log N_τ, were conflicting, and this prevented a definitive evaluation of the antimicrobial effect. For example, $T_{99.9\%}$ did not

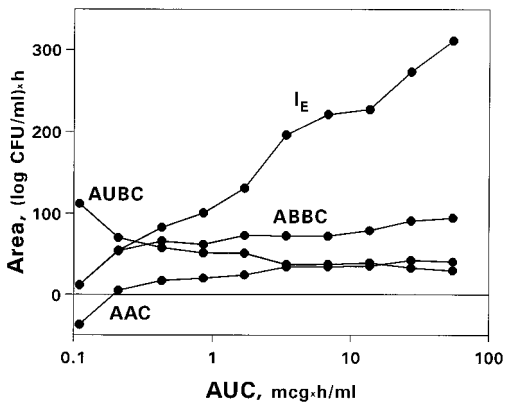

FIG. 5. AUC-induced changes in the integral parameters of bacterial killing and regrowth curves and the antimicrobial effect.

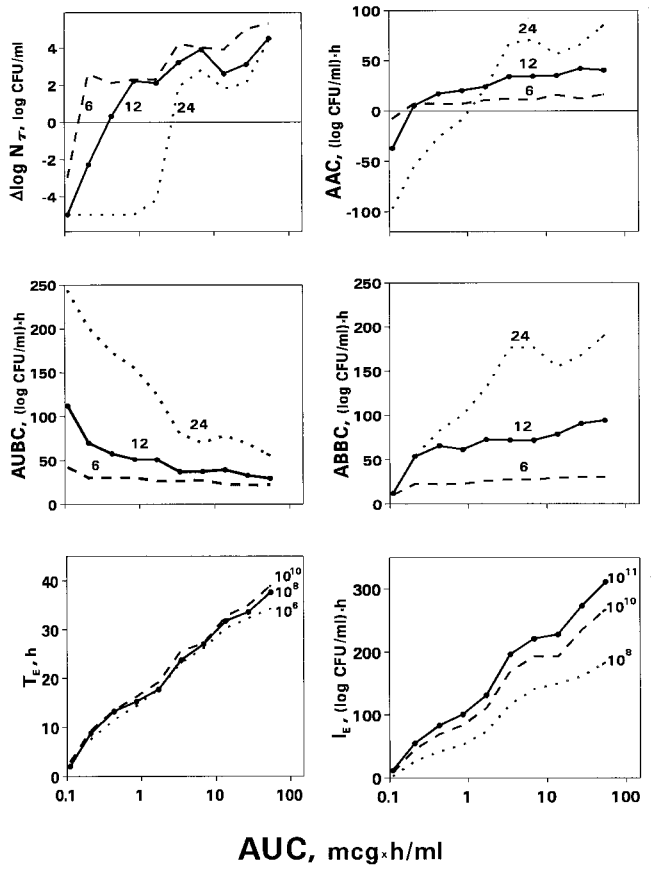

FIG. 6. Impact of cutting off the time-kill curves along the ordinate (N section; T_E and I_E) and the abscissa (τ section; $\Delta\log N_\tau$, AUBC, AAC, and ABBC) on parameter determination. The number at each curve corresponds to the N (in CFU per milliliter) or τ (in hours) cutoff level.

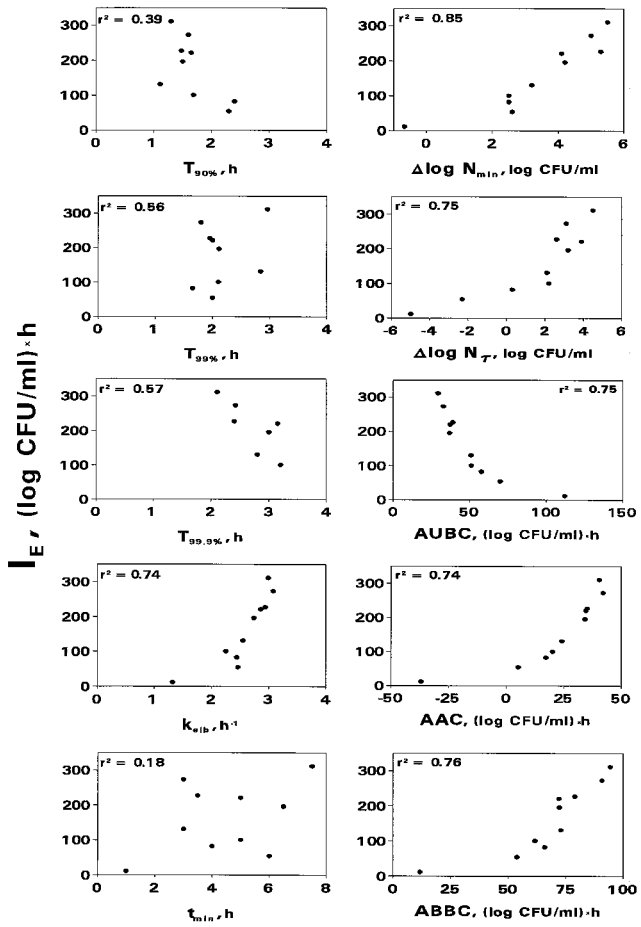

FIG. 7. Prediction of the total antimicrobial effect by different parameters. $\Delta\log N_\tau$, AUBC, AAC, and ABBC were estimated at a τ of 12 h.

correlate with $T_{90\%}$ ($r^2 = 0.16$) or $T_{99\%}$ ($r^2 = 0.19$), whereas $T_{90\%}$ correlated with $T_{99\%}$ ($r^2 = 0.87$). $T_{90\%}$ and $T_{99\%}$ correlated poorly with $\Delta\log N_{\min}$ ($r^2 = 0.35$ and 0.48) but correlated fairly well with $\Delta\log N_\tau$ ($r^2 = 0.58$ and 0.77), whereas $T_{99.9\%}$ correlated relatively well with $\Delta\log N_{\min}$ ($r^2 = 0.69$) but very poorly with $\Delta\log N_\tau$ ($r^2 = 0.16$). Unlike the parameters mentioned above, k_{elb}, AUBC, AAC, ABBC, T_E, and I_E correlated better with each other ($r^2 = 0.74$ to 0.99).

Robustness of the parameters and impact of study design. The irregular AUC-response relationships and/or uncertain interrelationships mentioned above may be considered indirect evidence of an insufficient robustness of $T_{90\%}$, $T_{99\%}$, $T_{99.9\%}$, t_{min}, $\Delta\log N_{min}$, and $\Delta\log N_\tau$. In addition, from general considerations, these parameters are less robust than k_{elb} and each of the integral parameters which consider not only separate points in the kinetic curves but also their sum total. However, the reliability of the k_{elb} estimates may depend on arbitrarily chosen quasilinear portions of the curvilinear ($\log N_A - \log N_C$)-time plots (see above), and the reliability of the AUBC, AAC, ABBC, I_E, and T_E estimates may depend on the procedure used for their determination and the experimental design (for the first three parameters only).

Figure 6 demonstrates the impact of cutting off the time-kill curves on the parameter determination. As seen in Fig. 6, AUBC, AAC, and ABBC, as well as $\Delta\log N_\tau$, were highly dependent on cutting off the time-kill curves at different times (τ sections). Depending on the τ section, even the pattern of the AUBC-, AAC-, ABBC-, and, especially, $\Delta\log N_\tau$-log AUC curves was liable to change. Unlike the τ- and design-dependent parameters, both T_E and I_E were less sensitive to the determination procedure. Although the estimates of these parameters depended on the upper level of viable counts in the regrowth and control growth curves (N section), the respective T_E- or I_E-log AUC curves had similar patterns. Moreover, I_E or T_E obtained at different N sections correlated well ($r^2 > 0.99$). Hence, these two parameters may be considered more robust than AUBC, AAC, and ABBC as well as $\Delta\log N_\tau$.

Prediction of the total effect by various parameters. As seen in Fig. 7, only one of the parameters of initial bacterial killing, k_{elb}, showed a reliable correlation with I_E, whereas $T_{90\%}$, $T_{99\%}$, and $T_{99.9\%}$ did not provide an accurate prediction of the total antimicrobial effect. Although t_{min} did not correlate with I_E, there was a fairly good correlation between $\Delta\log N_{min}$ and I_E ($r^2 = 0.85$). Less pronounced correlations were found between I_E and each of the four τ-dependent parameters measured at τ equal to 12 h ($r^2 = 0.74$ to 0.76). However, the predictive potential of $\Delta\log N_\tau$, AUBC, AAC, and ABBC could be enhanced by extending the observation period. For example, when measured at a τ of 24 h, the estimates of $\Delta\log N_\tau$, AUBC, AAC, and ABBC correlated with I_E much better ($r^2 = 0.88$ to 0.93) than those measured at a τ of 12 h and, especially, at a τ of 6 h ($r^2 = 0.68$ to 0.77). As seen in Fig. 7, distinct curvilinear patterns were noticeable in the I_E-$\Delta\log N_\tau$,

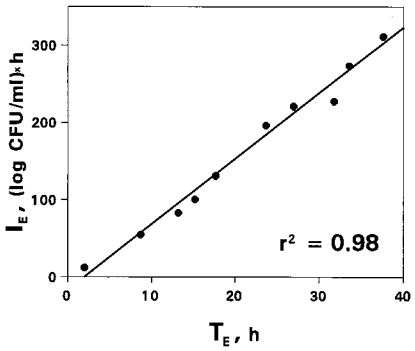

FIG. 8. Prediction of the total antimicrobial effect by T_E estimated at $N_C = N_A = 10^8$ CFU/ml.

-AUBC, -AAC, and -ABBC plots. Unlike τ-dependent parameters, the I_E-versus-T_E plot was quite linear (Fig. 8) and had the highest correlation coefficient ($r^2 = 0.98$).

DISCUSSION

Although numerous parameters have been described for the quantitation of time-kill curves or the antimicrobial effect as such, the data treatment and interpretation and the rational design of the in vitro studies remain problems. This study was aimed at direct comparison of the different parameters in terms of the AUC-response relationships since evaluation of such relationships is a primary goal of any pharmacodynamic and pharmacokinetic study. Twelve parameters, including those reflecting initial killing of bacteria ($T_{90\%}$, $T_{99\%}$, $T_{99.9\%}$, and k_{elb}), intermediate killing of bacteria (t_{min} and $\Delta \log N_{min}$), and total killing of bacteria ($\Delta \log N_\tau$, AUBC, AAC, ABBC, T_E, and I_E), were examined on the basis of time-kill studies with ciprofloxacin against *E. coli* in an in vitro dynamic model. The parameters were compared on the basis of four criteria: (i) relevance to be related to the AUC, (ii) sensitivity to the AUC, (iii) robustness, and (iv) ability to predict the total antimicrobial effect expressed by its intensity (I_E).

Unlike most parameters except T_E, I_E is not one more estimate of the time course of bacteria in the presence of an antibiotic but is a direct measure of the antimicrobial effect. By its very definition, I_E provides every point in the bacterial killing curve ($\log N_A$-t curve) to be referred to the respective point in the control growth curve ($\log N_C$-t curve), and thereby the dynamic pattern of the control is taken into account (10–12). This procedure differs from the ones providing a certain point in the $\log N_A$-t curve ($\Delta \log N_{min}$ and $\Delta \log N_\tau$) or a certain portion of this curve (AAC) to be referred to the initial inoculum $N(0)$: since bacteria not exposed to antibiotic do grow, $N_A(t)$ equal to $N_C(0)$ cannot be treated as the true baseline.

This study showed that the most regular relationships exist between I_E or T_E and the AUC of ciprofloxacin; moreover, I_E and T_E were the most sensitive to the changes in the AUC. Quite regular, although not quite reliable, relationships were established with AUBC, AAC, and ABBC. Depending on the duration of the observation period, the AUC-response curves were more or less degenerative. Furthermore, virtually negligible changes in AUBC, AAC, and ABBC observed at high AUCs might be treated as a certain saturation of the effect (Fig. 5) and not merely as being due to the apparent distortions of the logarithmic scale. The appearance of the plateau on the AUBC-, AAC-, and ABBC-log AUC curves at a large AUC was also reflected by insufficient sensitivity of these parameters to the AUC. Besides, low, if any, sensitivity of AAC to lomefloxacin doses (concentrations) mimicked in an in vitro model may be also noted, based on findings reported earlier (29).

Less regular relationships were established between AUC and the effect, as expressed by $\Delta \log N_{min}$, $\Delta \log N_\tau$, and k_{elb}, whose sensitivity to the AUC was intermediate and comparable to that of AUBC, AAC, and ABBC. Visual inspection of the time course of killing of bacteria exposed to pharmacokinetically consistent concentrations of lomefloxacin (5) shows that the concentration-dependent antimicrobial effect would probably be revealed by using any integral parameter as well as $\Delta \log N_{min}$ or $\Delta \log N_\tau$. At the same time, the slopes of the log N_A-time curves were almost insensitive to the simulated concentrations (no quantitation of the data has been done in the cited study). Dose- or concentration-dependent changes in N_{min} of *Klebsiella pneumoniae* exposed to ciprofloxacin in an in vitro dynamic model have also been reported (1).

Unlike k_{elb}, no reasonable AUC relationships could be established with $T_{90\%}$, $T_{99\%}$, $T_{99.9\%}$, and, especially, t_{min}, which were poorly sensitive to the AUC. It is remarkable that not only in our study but also in other studies k_{elb} appeared to be much more relevant than other measures of initial killing as an AUC-dependent parameter. Recently, no systematic dose-$T_{99.9\%}$ relationships were established when simulating the pharmacokinetic profiles of ciprofloxacin and lomefloxacin in an in vitro dynamic model (23). At the same time, relatively regular relationships have been reported between constant ciprofloxacin concentrations and the rates of killing of bacteria, as determined by k_{elb} (20, 21). However, a more sophisticated procedure of quantitation of initial killing of *E. coli* exposed to constant concentrations of ofloxacin, pefloxacin, and nalidixic acid (4) did not permit regular concentration-response relationships to be established.

As expected, $T_{90\%}$, $T_{99\%}$, and $T_{99.9\%}$, as well as other two point parameters, $\Delta \log N_{min}$ and $\Delta \log N_\tau$, yielded conflicting estimates of the time-kill curves and therefore prevented ultimate evaluation of the antimicrobial effect. Similar problems inherent in the quantitation of time-kill curves by means of poorly intercorrelated point parameters have been discussed earlier (13). Being highly vulnerable to possible errors of determination, these parameters cannot be considered robust; moreover, all of them are very dependent on the sampling schedule, replication, etc. Unlike the point parameters, k_{elb}, AUBC, AAC, ABBC, I_E, and T_E correlated better with each other. However, k_{elb} may be too sensitive to the quality of fitting of the curvilinear ($\log N_A - \log N_C$)-time plots, and AUBC, AAC, and ABBC are dependent on the cutoff level (τ section) of the log N_A-time curve. In fact, cutting off the time-kill curves resulted in underestimation of the effect expressed by these three integral parameters at high AUCs. Such an underestimation was more pronounced with a shorter τ. Hence, the design of time-kill studies may dramatically influence evaluation of the antimicrobial effect by use of AUBC, AAC, and ABBC.

Both I_E and T_E were free of the aforementioned shortcomings. Their estimates were almost insensitive to the level on the log N_A- and log N_C-time curves (N section) chosen for the determination of these parameters. The respective T_E- or I_E-log AUC curves had similar patterns, irrespective of the N section, and the I_E or T_E estimates obtained at different N sections correlated well. Hence, both parameters may be considered the most robust.

On the basis of the correlation coefficients, T_E was proven to be the best predictor and t_{min} was proven to be the worst predictor of the total antimicrobial effect expressed by I_E. The

predictive potentials of $\Delta \log N_{min}$, ABBC, AUBC, $\Delta \log N_{\tau}$, AAC, and k_{elb} appeared to be reasonably high, whereas those of $T_{99\%}$, $T_{99.9\%}$, and, especially, $T_{90\%}$ were low. Again, the predictability of I_E by AUBC, AAC, ABBC, and $\Delta \log N_{\tau}$ was dependent on τ. It is by no means accidental that the predictive potentials of AUBC, AAC, and ABBC, but not that of $\Delta \log N_{\tau}$, could be enhanced by using a more protracted observation period.

Thus, only I_E and T_E met all four criteria of an optimal parameter of the antimicrobial effect, although I_E was superior to T_E in terms of sensitivity to the AUC, whereas T_E was superior to I_E in terms of sensitivity to the determination procedure. However, I_E is preferable to T_E for at least two reasons. (i) Unlike T_E, I_E, being an integral parameter, is determined not only by two points on the regrowth and normal growth curves but by all the points, and (ii) I_E, unlike T_E, is able to reflect multiple increases or decreases in the viable counts produced by repeated antibiotic administration. So, I_E is a more universal measure of the antimicrobial effect.

In conclusion, this study demonstrates feedback relationships between the design of time-kill experiments and their quantitation. Depending on the duration of the observation period and the parameter used, the very idea of an AUC-, dose-, or concentration-related antimicrobial effect may be more or less biased. In general, the most unbiased presentation of the effect may be obtained when operating with full-term log N_A- and log N_C-time curves, including the regrowth phase, and with the I_E or T_E parameter. Unjustified shortening of the observation period, i.e., cutting off the log N_A-time curves along the time axis, may lead to misrepresentation of the relationships between the effect and the pharmacokinetic variable(s), to a point where it gives rise to the false idea of an AUC-, concentration-, or dose-independent effect. In this regard concentration-independent initial killing does not mean that the total antimicrobial effect is also concentration independent. Moreover, the total effect often cannot be accurately predicted by initial killing of bacteria. However, conclusions based on killing rate estimates in short-term experiments are often automatically expanded to the total effect. It is worthy of note that, despite the fact that the widespread notion about concentration-independent effects of β-lactam antibiotics has become something of a cliché, distinct AUC- or concentration-dependent changes in the T_E of cefotaxime (8, 9) and ceftizoxime (28), although less pronounced than those in the I_E of aminoglycosides (7) and fluoroquinolones (this study), have been established. Thus, the design of time-kill studies and their quantitation may be decisive for our knowledge of antimicrobial agents.

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