Development of a New Quantitative Approach for the Isobolographic Assessment of the Convulsant Interaction Between Pefloxacin and Theophylline in Rats

Laurence M. Levasseur,[1] Annie Delon,[2] William R. Greco,[1] Pascal Faury,[2] Serge Bouquet,[2] and William Couet[2,3]

Received February 3, 1998; accepted April 14, 1998

Purpose. A new mathematical approach was developed to quantify convulsant interaction between pefloxacin and theophylline in rats.
Methods. Animals received each compound separately or in different combination ratios. Infusion was stopped at the onset of maximal seizures. Cerebrospinal fluid (CSF) and plasma samples were collected for HPLC drug determination. The nature and intensity of the pharmacodynamic (PD) interaction between drugs was assessed with a new modeling approach which includes (a) data transformation to create an essentially error-free X-variable and (b) estimation of an interaction parameter α by fitting a nonlinear hyperbolic model to the combination data with unweighted nonlinear regression.
Results. Drug disposition to the biophase was linear within the range of administered doses. The estimates of α suggested a Loewe antagonistic interaction between pefloxacin and theophylline at the induction of maximal seizures in rats. Similar intensity of PD interaction was observed at the dose and biophase level (α was -0.415 ± 0.069 and -0.567 ± 0.079, respectively).
Conclusions. The suitability of the proposed model was assessed by Monte Carlo simulation. This new mathematical approach enabled the characterization of the Loewe antagonistic nature of the PD (convulsant) interaction between pefloxacin and theophylline, whereas previously used methodologies failed to do so.

KEY WORDS: quinolones; seizures; pharmacodynamics; nonlinear hyperbolic model; combination index; Monte Carlo simulation.

INTRODUCTION

Pharmacokinetic (PK) interactions between quinolones, including pefloxacin, and theophylline, have been frequently described (1). However, because both quinolones and theophylline exhibit central nervous system (CNS) excitatory effects, possibly leading to convulsions, one should also be aware of a potential PD interaction between quinolones and theophylline. The convulsant activity of theophylline has been investigated *in vivo* in rats together with measurements of the drug concentrations in the biophase. This approach enabled the isolation of the PD of the convulsant effect of the drug from its PK characteristics (i.e., ability to reach its pharmacological receptors at the CNS level) (2). A similar approach has been used to elucidate the PD interactions between theophylline and caffeine or pentylenetetrazol (3). We have recently investigated the PD contribution to the convulsant activity of two quinolones, pefloxacin and norfloxacin (4); we now propose to investigate the PD interactions between pefloxacin and theophylline with a new approach for combined-action assessment.

MATERIALS AND METHODS

Animals

This work was done in accordance with the *Principles of Laboratory Animal Care* (NIH Publication #85-23, revised 1985). Male Sprague Dawley rats (n = 54) from Depres Breeding Laboratories (St. Doulchard, France), were housed in the Animal Breeding Facilities of the Laboratory (authorization N° 0028). Their mean ($\pm$ SE) body weight was equal to 240 ± 2 g. The animals were placed in wire cages in a 12 hours light-dark cycle for one week with free access to food (Extra-labo M20, Pietrement Laboratories, France) and water.

Surgery

Surgery was as previously described (4), except that because of physical incompatibilities between the two drug solutions leading to precipitation, two polyurethane catheters (0.51-mm inside; 0.71-mm outside diameter, Plastimed Laboratories, France) were inserted in the jugular vein. The risk of precipitation in blood at the site of injection was minimized by leaving a gap between the extremity of each catheter. When only one drug was infused (pefloxacin or theophylline), only one polyurethane catheter (0.58-mm inside; 0.98-mm outside diameter, Plastimed Laboratories, France) was used.

Solutions for Administration

The drugs were administered as: 1) an 80 mg/mL (240 mM) commercially available solution of pefloxacin methane sulfonate (Bellon Laboratories, France); and 2) a 25 mg/mL solution of aminophylline (corresponding to 19.7 mg/mL, or 109 mM, of theophylline base) for intramuscular or intravenous administration (Assistance Publique des Hôpitaux de Paris, France).

Drug Administrations and Sample Collection

The day after surgery, the jugular vein cannulas were connected to a 2-way motor-driven syringe pump (SE200B, Vial Inc., France) equipped with two syringes containing pefloxacin solution for one, and theophylline for the other. Flow rates of each syringe were adjusted in order to achieve the desired rate

[1] Roswell Park Cancer Institute, Biomathematics Department, Buffalo, New York.
[2] UPRES 1223, Faculté de Médecine & Pharmacie, Poitiers, France.
[3] To whom correspondance should be addressed. (e-mail: William.Couet@campus.univ-poitiers.fr)

ABBREVIATIONS: PK, pharmacokinetic; PD, pharmacodynamic; CNS, central nervous system; CSF, cerebrospinal fluid; UF, ultrafiltrate; HPLC, high performance liquid chromatography.

0724-8741/98/0700-1069$15.00/0

of drug delivery (Table I). The total flow rate was equal to 4 mL/hr. Animals were kept under a heating lamp to maintain body temperature. The infusion was stopped when the animals exhibited maximal seizures. Onset of maximal seizures was usually evidenced by tonic flexion of the forelimbs and tonic extension of the hindlimbs. The total infused volume ranged between 1.60 and 4.00 mL. Drug administration was conducted between 2:00 p.m. and 6:00 p.m. CSF and plasma samples collection was as previously described (4).

Drug Analysis

Pefloxacin and theophylline concentrations were determined simultaneously by HPLC using a previously described methodology (4,5) with minor modifications including UV detection at 280 nm. Retention times of pipemidic acid (internal standard), theophylline and pefloxacin were respectively 5.2, 6.5 and 12.2 min. HPLC repeatability measurements of quality control samples showed that the analytical error was equal to or less than 5%.

Data Analysis

The structure of the error in the data was modeled with Eq. 1, which implies that the variances, s_A^2, are linearly related to means, $\overline{X}_A$, on log-log coordinates (6). For each set of replicates of pairs of (X,Y) data, Eq. 1 was fit to the log-log transformed data with unweighted linear regression. By replicates, we considered (a) the HPLC measurements of total plasma concentrations (Cp), UF concentrations (Cu) or CSF concentrations (Ccsf), for pefloxacin or for theophylline, at each fixed ratio of pefloxacin dose to theophylline dose (3–6 replicates per ratio) or (b) the combination indexes (defined below) calculated for each fixed ratio of pefloxacin dose to theophylline dose. A constant coefficient of variation is indicated when the estimated slope of the line, ϕ_3 is found to be close to 2; the parameter ϕ_2 is then equal to the square of the coefficient of variation. For subsequent fittings of models to pairs of (X,Y) data with nonlinear regression, data were weighted by the reciprocal of the predicted dependent variable raised to their power ϕ_3.

$$s_A^2 = \phi_2 \overline{X}_A^{\phi_3} \quad (1)$$

For each of the drugs, the relationship between Ccsf and infused dose was determined by fitting Eq. 2 to data with iteratively reweighted nonlinear regression. In Eq. 2, X is the dose of pefloxacin, or theophylline; Y is the corresponding CSF concentration; β_1 is the slope of the linear relationship; and β_2 is a curvature factor. When the parameter estimate of β_2 is significantly different from zero, a nonlinearity among dose and concentration is indicated.

$$Y = \beta_1 X + \beta_2 X^2 \quad (2)$$

The dose-dependency at the level of the unbound plasma fraction (fu) and Ccsf/Cu ratio were studied for each drug. In Eq. 3, X is the dose of pefloxacin (or theophylline); Y is fu, or the Ccsf/Cu ratio of pefloxacin (or theophylline); β_0 is the intercept parameter; and β_1 is the slope of the linear relationship between Y and the dose of pefloxacin (or theophylline). When the parameter estimate of β_1 is significantly different from zero, a dose-dependence of fu, or Ccsf/Cu, is suggested.

$$Y = \beta_0 + \beta_1 X \quad (3)$$

A plausible model for the isobol (Eq. 4) has been previously derived (7). In Eq. 4, for drug 1 and drug 2, C is the dose, or concentration, of drug in combination required to induce maximal seizures in rats, and $\overline{IC}$ is the geometric mean dose, or concentration, of drug which when given alone was required to induce maximal seizures. Note that for experiments with typical continuous or binary (yes/no) biological responses, $\overline{IC}$ can be replaced by IC_{50}, or EC_{50}, commonly defined as the concentration (or dose) which results in 50% of the maximal response, or which results in 50% of the experimental subjects exhibiting a response, respectively. The definition of $\overline{IC}$ used for this direct assay is a measure of central tendency of the tolerance distribution for the induction of seizures. The interaction parameter is α (7). The absolute magnitude of α is directly related to the degree of bowing of the isobol. When α is positive, Loewe synergy is indicated, whereas a negative value of α reflects Loewe antagonism. The interaction is concluded to be Loewe additive if the 95% confidence interval for α encompasses zero.

$$\frac{\frac{C_2}{\overline{IC}_2} = \frac{1 - \frac{C_1}{\overline{IC}_1}}{1 + \alpha \frac{C_1}{\overline{IC}_1}}}{} \quad (4)$$

However, we chose not to fit the isobol model to data because both X- and Y-variables are random variables and thus, subject to error. For a direct assay, the output is the dose, or concentration, of drug inducing a specific effect, for instance the onset of maximal seizures. Therefore for interaction studies, the data consist of pairs of doses, or concentrations, (C_1,C_2) for each of the two agents, in which both C_1 and C_2 are random variables subject to error. All sources of random error encountered at the dose, plasma and CSF level in this particular direct assay are illustrated in Fig. 1. The onset of maximal seizures is directly related to the concentration of the drug in the biophase

Table I. Summary of Experimental Conditions of the Interaction Study

Drug combination				
Pefloxacin: theophylline ratio[a]	Pefloxacin: theophylline ratio[b]	Number of animals	Body weight (g)	Infusion time (min)
4.0:0.0	∞	4	245 ± 7	26.7 ± 0.6
3.6:0.4	19.8	4	240 ± 13	25.4 ± 0.7
3.2:0.8	8.8	5	239 ± 15	29.1 ± 1.4
2.8:1.2	5.1	5	239 ± 11	30.5 ± 2.6
2.4:1.6	3.3	5	249 ± 10	38.9 ± 4.1
2.0:2.0	2.2	5	237 ± 10	34.6 ± 2.4
1.6:2.4	1.5	8	244 ± 6	41.4 ± 2.4
1.2:2.8	0.9	5	239 ± 4	44.8 ± 3.0
0.8:3.2	0.6	3	230 ± 11	51.2 ± 5.3
0.4:3.6	0.2	5	241 ± 7	49.0 ± 4.6
0.0:4.0	0	5	237 ± 8	48.5 ± 2.3

Note: Data are presented as mean ± SE.
[a] Ratio of flow rates; the total flow rate was constant at 4.0 mL/hr.
[b] Ratio of input rates in molar units.

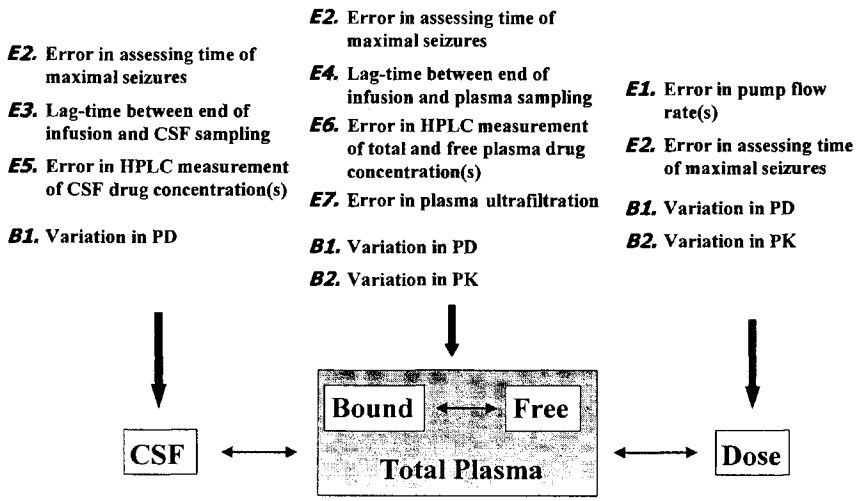

Fig. 1. Schematic representation of the main sources of random experimental (E1–E7) and biological (B1, B2) error in the direct assay at the CSF concentration level, free and total plasma concentration level and at the dose level.

(CSF). Therefore, Fig. 1 should be read from left to right, from CSF to dose.

Because both the Y-variable (C_2) and the X-variable (C_1) will contain similar amounts of error, common regression techniques, which assume that the independent variable is measured without appreciable error, should not be used (8). Also, in an isobologram, the Y-variable is only indirectly caused by the X-variable. In order to enable the use of standard regression approaches for this problem, and to arrange for the X-variable to 'cause' the Y-variable in a logical manner, a new modeling approach based upon a data transformation was developed as detailed below.

By manipulating Eq. 4, one can easily express the combination index (CI) as a function of the proportion of pefloxacin (R) in the mixture, in terms of $\overline{IC}$ equivalents (Eq. 5). Note that R can be written in terms of r ($r = C_2/C_1$), the fixed ratio of drug 2 (theophylline) dose, or concentration, to drug 1 (pefloxacin) dose, or concentration, and in terms of the mean potencies $\overline{IC}_1$ and $\overline{IC}_2$ (random variables). If the number of data points, N, that contribute to the estimation of $\overline{IC}_1$ and $\overline{IC}_2$ is large, the variance of the estimates will be small. Therefore, for large N, R will be relatively free of error, and most of the variation will be in the Y-variable, CI. This is perfectly true when doses are used for the combination index plot, but less true when concentrations of drugs measured in biological fluid (plasma, UF or CSF) are used. In contrast, CI will always include the dose (or concentration) of the injected combination, the true random variable for this direct assay.

Since Eq. 5 is not in closed form, a one-dimensional bisection root finder was used to calculate predicted values of CI. Note that one can also solve Eq. 5 for CI, and fit the equation of the positive root to the data. When CI is greater than 0, but less than 1, Loewe synergy is indicated (positive value of α); when CI is greater than 1, Loewe antagonism is indicated (negative value of α); and when CI is not significantly different from 1, the combination is Loewe additive (α is not significantly different from 0). Although the combination index concept has been extensively used in the past by Berenbaum (9) and others, it has been adapted here for a direct assay.

$$\alpha\, R(1 - R)CI^2 + CI - 1 = 0 \qquad (5)$$

where

$$R = \frac{\frac{C_1}{\overline{IC}_1}}{\frac{C_1}{\overline{IC}_1} + \frac{C_2}{\overline{IC}_2}} = \frac{\overline{IC}_2}{\overline{IC}_2 + \overline{IC}_1 \frac{C_2}{C_1}} \text{ and } CI = \frac{C_1}{\overline{IC}_1} + \frac{C_2}{\overline{IC}_2}$$

To illustrate the relationship between the value of α and the degree of bowing of the isobol, as well as the relationship between Eq. 4 (isobologram plot) and Eq. 5 (CI plot), simulations were performed and are displayed in Fig. 2 for values of α ranging from -0.5 to 10. Note that inherent to the structure of the interaction model, the magnitude of the parameter α does not translate to the same degree of bowing for synergism and antagonism. A simulation of the theoretical isobol has shown that similar bowings were obtained for α values of 100 (Loewe synergy) and -0.99 (Loewe antagonism) (see Figure 6 in (7)). The Loewe additivity is represented by the diagonal line in the isobol plot, or by the horizontal line at the ordinate CI = 1 in the combination index plot. If the experimental points fall below

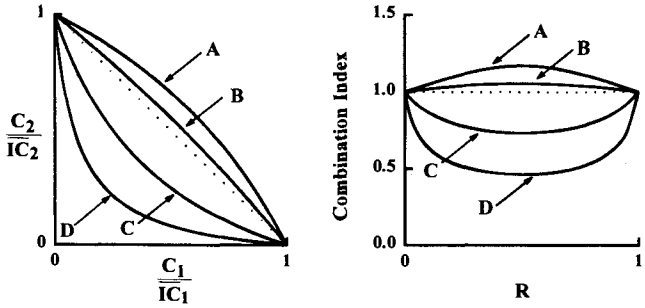

Fig. 2. Isobologram plot (left panel) and corresponding combination index plot (right panel). The dashed line is the theoretical additivity line. The solid curves are the isobol and combination index simulated from Eq. 4 and Eq. 5, respectively, for values of the interaction parameter α of -0.5 (curve **A**), -0.2 (curve **B**), 2 (curve **C**) and 10 (curve **D**).

the additivity line, the combination is considered Loewe synergistic ($\alpha > 0$, or CI < 1), whereas points above the additivity line suggest a Loewe antagonistic combination ($\alpha < 0$, or CI > 1) (7).

The suitability of Eq. 5 as a candidate model for assessing drug interaction in a direct assay was studied with an extensive Monte Carlo simulation. Data (45 points per data set; 7 fixed ratios of drug doses [ratios 1:0, 10:1, 4:1, 2:1, 1:1, 1:2, 1:4, 1:10 and 0:1]; 5 replicates) were generated using Eq. 5 for 3 values of the interaction parameter (antagonism, $\alpha = -0.5$; additivity, $\alpha = 0$; synergy, $\alpha = 10$). Mean drug potencies were set at biologically relevant doses; i.e., 1750 and 1500 for drug 1 and 2, respectively. The error was assumed to follow either a normal or a log-normal distribution. Data sets (1000) containing random error were generated for each of the 6 situations (i.e., 2 error models × 3 interaction levels) with a random number generator using Eq. 1 and assuming a constant coefficient of variation of 12.2% ($\phi_2 = 0.015$; $\phi_3 = 2$). Each of the 1000 data sets was analyzed by fitting the combination index model (Eq. 5) and the isobol model expressed as a function of drug 1 or drug 2 (Eq. 4) with weighted nonlinear regression.

The weighing scheme was formed as explained above by fitting Eq. 1 to sets of mean-variance data from sets of replicates. In order to define the best weighing approach for the fitting of Eq. 5 to the CI data, nonlinear regressions were also performed for ϕ_3 set at 0 (no weighing) and 2 (constant coefficient of variation). For each of the 6 cases (2 error distribution models × 3 interaction intensities), the distributions of the final parameter estimates of α were studied for precision and biases among the 5 different data analysis approaches (2 isobol models + 3 weighting techniques for the CI model). A total of 30,000 fits of data were performed with nonlinear regression for this Monte Carlo study.

The fitting of linear and nonlinear models to data was performed with PROC NLIN in SAS (10), release 6.11 for Windows, using the multivariate secant method (11). Error-containing data sets for the Monte Carlo simulation were generated with a random number generator implemented in SAS. Significance of the parameters was assessed by forming the 95% confidence interval of the estimates. Parameters were considered significant if their confidence interval excluded zero. All graphs were prepared with Sigmaplot, Ver. 4.0 (SPSS, Chicago, IL). Software was run on Pentium-based microcomputers. Results are expressed as mean parameter ± SE.

RESULTS

A total of 54 rats was used in this study. However, technical problems such as failure to determine the infusion time or to obtain plasma UF, as well as blood contamination of CSF samples, accounted for the loss of several values. Therefore, experimental data for doses, Cp, Cu, and Ccsf, were obtained from 52, 50, 45, and 49 animals respectively. Under these experimental conditions, maximal seizures occurred within 25.4 to 51.2 min on average, depending on the composition of the infusion solution (Table I).

In agreement with previous experiments conducted with a similar approach (2,4), the concentrations of theophylline and pefloxacin metabolites in plasma and CSF were below assay limits in these experiments, and their contribution to the convulsant activity was neglected.

The relationship between Ccsf and dose is displayed in Fig. 3 for pefloxacin (A) and theophylline (B). Different symbols were used for each fixed ratio of pefloxacin to theophylline dose. The modeling of the error distribution was performed with Eq. 1 separately for each set of concentration-dose data; means and variances were calculated from replicates of each ratio. ϕ_3 was 2.02 for pefloxacin and 0.776 for theophylline. Eq. 2 was fit to data with weighted nonlinear regression; data were weighed by the reciprocal of the predicted variable raised to the power ϕ_3. Since both drugs exhibited a linear relationship between Ccsf and dose (β_2 not significantly different from zero), Eq. 2 was simplified by setting β_2 to 0. Parameter estimates of β_1 were 0.192 ± 0.012 $(L/kg)^{-1}$ for pefloxacin and 0.797 ± 0.0095 $(L/kg)^{-1}$ for theophylline.

For the two drugs, fu was independent of the infused dose (β_0 was 0.769 ± 0.012 and 0.733 ± 0.025 for pefloxacin and theophylline, respectively; β_1 was not significantly different from zero; Eq. 3). The Ccsf/Cu ratio was also dose-independent for pefloxacin ($\beta_0 = 0.641 \pm 0.026$). For theophylline, the parameter β_1 was significantly different from zero and suggested a slight decrease of Ccsf/Cu as dose increased ($\beta_0 = 1.36 \pm 0.16$ and $\beta_1 = -0.000458 \pm 0.00021$ $(\mu mol/kg)^{-1}$).

The parameter estimates of the mean potencies of pefloxacin, $\overline{IC}_1$, and theophylline, $\overline{IC}_2$, along with 95% confidence intervals are reported in Table II. The ratio of mean potency of theophylline to pefloxacin at the level of doses, Cp, Cu, or Ccsf were 0.857, 2.45, 2.23, and 3.57, respectively. Therefore, on the basis of CSF concentrations, the previously defined intrinsic convulsant activity (4) of pefloxacin is 3.57 fold higher on average than that of theophylline. The ratio values for Cp and Cu are virtually identical, and somewhat lower than the ratio of CSF concentrations, which can be explained by the difference in the ability of these two drugs to cross the blood-cerebrospinal fluid barrier. The considerably lower ratio value based on doses reflects differences in the distribution kinetics (i.e., apparent volume of distribution) of pefloxacin and theophylline (3).

Fig. 4 presents the distribution of the estimate of the interaction parameter α generated by fitting nonlinear models (Eq.

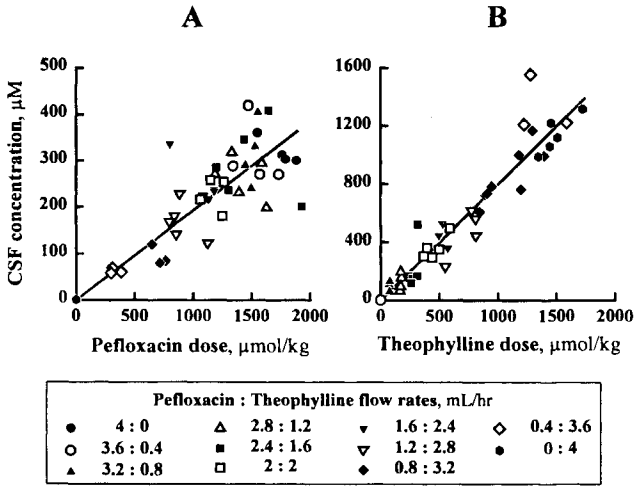

Fig. 3. Relationship between CSF concentration and dose of pefloxacin (**A**) and theophylline (**B**). The solid curves are the best fit of Eq. 2 to data by weighted nonlinear regression.

Table II. Mean Potency (Geometric Average) for Pefloxacin ($\overline{IC_1}$) and Theophylline ($\overline{IC_2}$) When Each Was Given Alone, Followed by the 95% Confidence Interval into Brackets

	$\overline{IC_1}$	$\overline{IC_2}$	α
Dose	1744 [1609–1890]	1494 [1380–1618]	− 0.415 ± 0.069
Plasma	705 [534–930]	1729 [1361–2196]	−0.502 ± 0.072
UF	567 [502–641]	1263[a]	−0.480 ± 0.085
CSF	318 [292–345]	1134 [1026–1253]	−0.567 ± 0.079

Note: Eq. 5 was fit to pairs of (R, CI) data with unweighted nonlinear regression enabling the estimation of the interaction parameter α, along with SE. Doses are in μmol/kg and plasma, UF and CSF concentrations are in μM.

[a] Only one determination available

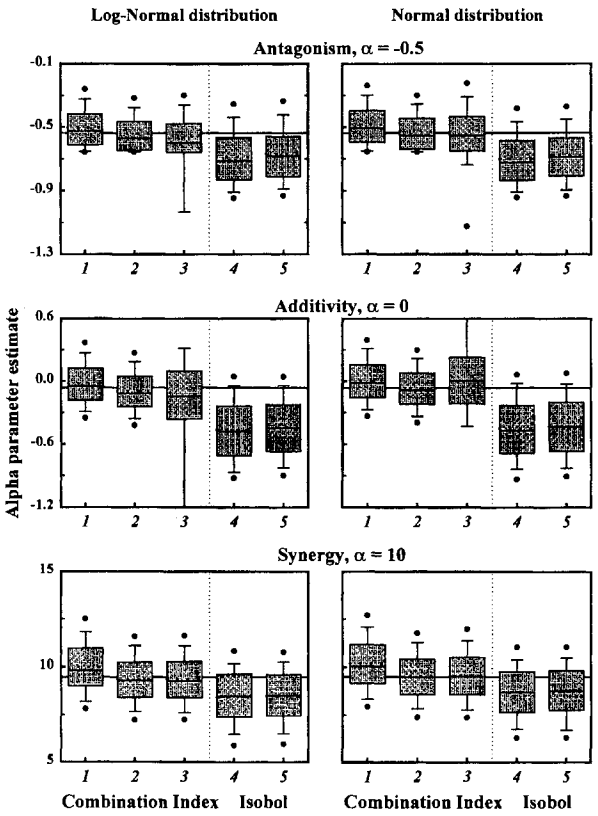

Fig. 4. Box-plot representation of the distribution of the interaction parameter α obtained through the Monte Carlo simulation of 1000 data sets. Log-normally (left panels) or normally (right panels) distributed random error was added to data simulated from Eq. 5 for antagonistic (true α = −0.5, top panels), additive (true α = 0, middle panels) or synergistic (true α = 10, bottom panels) interactions. Mean drug potencies were 1750 and 1500 for drug 1 and 2, respectively. Error was generated with Eq. 1 assuming a constant coefficient of variation. The five approaches for assessing drug interaction are defined as follows: the CI model (Eq. 5) was fit to data with nonlinear regression, unweighted (box **1**), weighted by the reciprocal of the squared predicted CI (box **2**), or weighted by the reciprocal of the predicted CI raised to the power ϕ_3 (box **3**); the isobol model (Eq. 4) was fit with nonlinear regression to pairs of drug 1 - drug 2 data (box **4**), weighted by the reciprocal of the predicted drug 1 dose raised to the power ϕ_3, or pairs of drug 2 - drug 1 data (box **5**), weighted by the reciprocal of the predicted drug 2 dose raised to the power ϕ_3. The box-plot features the median (solid line), mean (dotted line), inter-quartile range (gray box), 10^{th}–90^{th} percentile (error bar) and 5^{th}–95^{th} percentile (black circle).

4–5) to data simulated by the Monte Carlo technique. Because dose or concentration data tend to be log-normally distributed, the discussion of the results will emphasize the comparison of the 5 procedures applied to data containing a log-normal error. However, note that similar tendencies in terms of precision and bias exist in the left panel (log-normally distributed error) and right panel (normally distributed error). The first 3 boxes in each panel shows the distribution of α when the CI model (Eq. 5) was fit to data with nonlinear regression. Data were unweighted (box 1), or weighted by the reciprocal of the predicted CI raised to the power 2 (box 2) or ϕ_3 (box 3). It is clear from Fig. 4 that this third weighting approach yielded the least precise and most biased estimation of α, for antagonistic and additive interaction. For synergistic interaction, the 3 weighting techniques resulted in similar precision.

The precision of the first 2 statistical approaches was comparable; however, the fitting of Eq. 5 with unweighted regression (box 1) led to the least biased estimation of α (e.g., the coefficient of variation of the α parameter estimates was 25.3% and 21.0%, for boxes 1 and 2 in the top left panel, but the percentage of bias was 0.583% and 8.30%, respectively). In agreement with these results, for our subsequent fitting of Eq. 5 to real laboratory data with nonlinear regression, the data were unweighed.

Parameter estimates of α obtained with Eq. 4 are also shown in each panel (boxes 4 and 5). The fit of the isobol model to pairs of drug 1 - drug 2 data (box 4) or pairs of drug 2 - drug 1 data (box 5) yielded similar distributions of α estimates. Precision in α was essentially comparable with that obtained with the CI model (CV's were 26.7% and 27.8% for boxes 4 and 5 in the top left panel), but large biases were observed (percentages of bias were 38.0% and 33.6% for boxes 4 and 5 in the top left panel) leading to a systematic underestimation of the absolute magnitude of the intensity of interaction.

The final model (Eq. 5) was fit to pairs of real laboratory (R,CI) data with unweighed nonlinear regression. The transformed data and the fitted model are shown in Fig. 5 for the 4 sets of data, and the corresponding α parameters with accompanying standard error are displayed in Table II. Overall, the intensity of the drug interaction was not significantly different at the level of the dose, Cp, Cu or Ccsf ($p > 0.05$). The estimates of α, in particular −0.415 ± 0.069 for doses and −0.567 ± 0.079 for CSF levels, suggest a Loewe antagonistic interaction between the two drugs. The isobolograms for doses, Cp, Cu and Ccsf are displayed in Fig. 6. The simulated curves in Fig. 6 represent the same fitted curves as in Fig. 5 re-transformed back to the isobologram coordinates.

Note that corresponding increases in the doses and CSF levels of each of the two drugs at the onset of activity, compared to the additivity prediction, can be calculated for any value of R. The maximum percentage of change corresponds to a mixture with equi-proportion of pefloxacin and theophylline. If this given R (i.e., 0.5) is substituted in Eq. 5, along with the proper value of α (i.e., −0.415 for dose, or −0.567 for CSF), one

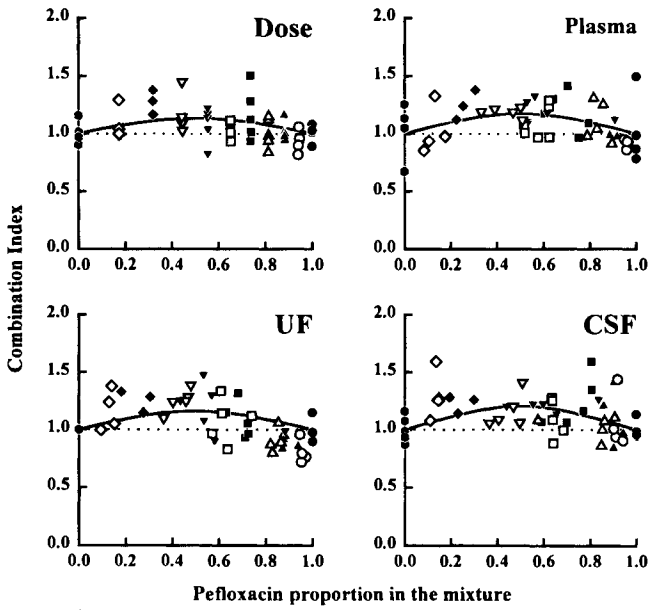

Fig. 5. Combination index versus pefloxacin proportion in the mixture for the injected dose, and the corresponding plasma, UF and CSF concentrations. The dashed line is the theoretical additivity line. The solid curve was obtained by fitting Eq. 5 to the data.

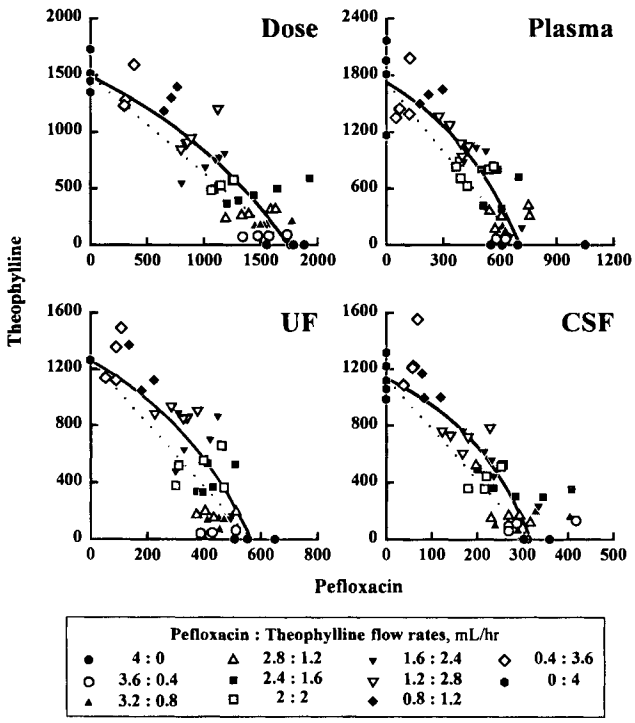

Fig. 6. Isobolographic representation of the convulsant interaction between pefloxacin and theophylline in rats. Each point is the actual injected dose of pefloxacin and theophylline alone or in combination, and the corresponding plasma, UF and CSF concentrations. Dose is in μmol/kg, and concentrations are in μM. The dashed line is the theoretical isobol for additivity. The solid line is the isobol simulated with Eq. 4 for values of the interaction parameter α obtained from fitting Eq. 5 to the data.

would obtain a value for CI of 1.13 for dose, or 1.21 for CSF, that can be compared to the additivity prediction (i.e., CI = 1). Therefore, under these conditions, a 13% increase in the dose, or a 21% increase in the CSF concentration, of each of the two drugs, is necessary to produce the onset of maximal seizure.

DISCUSSION

Data obtained in this study for pefloxacin or theophylline, when administered alone, compare favorably with literature data. The mean convulsant dose of pefloxacin (1744 μmol/kg) is within the range of values previously reported (4). The geometric mean Ccsf at the onset of maximal seizures (318 μmol/L) seems to be reduced compared to the previous estimate (380 ± 5 μmol/L) (4), but it is virtually identical to a value of 323 ± 9 μmol/L recently obtained in another experiment (12). Such differences are consistent with inter-occasion variability (4). For theophylline, the mean CSF concentration (1134 μmol/L) was close to the value of 232 ± 3 μg/mL (corresponding to 1288 ± 15 μmol/L) previously published (2).

Zhi and Levy (3) previously published a data analysis approach to the same problem addressed in this report. Their approach to assessing interaction between two agents in a direct assay included: (a) estimating the mean dose and 95% confidence interval for each agent when given alone; (b) constructing a theoretical Loewe additivity line and a confidence envelope by connecting the respective mean and lower and upper limit values of each drug when given alone; (c) fitting the data on the isobologram for 2 agents applied in combination with a straight line via a 'bivariate linear regression analysis assuming equal error in both variables'; and (d) assessing whether the combination data points lie within the bounds of the 95% confidence envelope for the Loewe additivity line. The construction of the confidence interval for the Loewe additivity prediction is derived from an approach proposed by Gessner (13). It has the limitation of being highly conservative; small departures from Loewe additivity are unlikely to be detected. The specific statistical methodology used for fitting the combination data is neither described nor referenced (3). It is also not clear how the fitted line is used in making inferences about Loewe synergy or Loewe antagonism. Furthemore, this approach was not able to capture the antagonistic nature of the interaction between pefloxacin and theophylline.

In contrast, the new modeling approach developed in this report is based on the fitting of a nonlinear hyperbolic model to the combination data with unweighted nonlinear regression. The model (Eq. 5) follows the shape of classical hyperbolic Loewe synergistic and Loewe antagonistic isobols, whereas the linear model of Zhi and Levy does not. An interaction parameter is estimated along with an uncertainty measure, which characterizes not only the nature of the drug interaction but also its intensity. The Monte Carlo simulation proved that the combination index model was superior to the fitting of isobols in assessing the intensity of drug interaction in a precise and relatively unbiased manner. In addition, it was shown that biases in the parameter estimates were least when Eq. 5 was fit to data with unweighed rather than weighed nonlinear regression.

Note that, instead of Eq. 5 written in an unclosed form, the positive root could have been fit to the CI data. Since no advanced nonlinear regression features (e.g., loop for the root finder method, weighting procedures) are necessary, the modeling technique introduced in this report can be easily implemented in any standard package (e.g., SigmaPlot, PCNonLin, Adapt II, SAS).

But essentially, the data transformations in Eq. 5 facilitate the use of standard regression techniques (8) which assume that the X-variable does not contain error. This is a reasonable assumption when doses of drugs are used for constructing the combination index plot, but less true when concentrations of drugs measured in biological fluids are used. As the number of replications of the single agent determinations increases, the assumption that the proportion of drug 1 (X-variable) contains no error becomes more reasonable; most of the variation will be in the Y-variable CI. The requirement of error-free X-variable is obviously not fulfilled with the regular isobologram approach (13), since an appreciable error exists in C_1 (X-variable), and this error is correlated to the one existing for C_2 (Y-variable).

For a fixed ratio combination, the variability in C_2 and C_1 is in a perpendicular direction to the fitted isobol and runs along a ray between the origin and the isobol (see Fig. 6 Dose panel). C_1 and C_2 are perfectly positively correlated; if one knows the fixed ratio of C_2 to C_1 (usually fixed by the experimenter) and the amount of C_1 (or C_2) in the mixture that elicits the specified effect, then one can directly calculate the amount of C_2 (or C_1) in the mixture. This type of error structure implies that regression approaches which minimize a function of the perpendicular distance between the data points and the fitted curve may be appropriate for this type of problem.

In addition, the isobolographic representation and the subsequent fitting of a model expressing C_2 as a function of C_1 (e.g., Eq. 4), inherently implies that C_2 is 'caused' by C_1, which is not exactly true. We propose here the transformation of the standard isobologram coordinates (C_1,C_2) to the pair of variables (R,CI). This new relation implies that the X-variable R, the proportion of drug 1 in the mixture in potency equivalents, 'causes' the Y-variable CI, the measure of the intensity between the drugs.

Equation 5 was examined with the SAMPLE schedule design program in ADAPT II (14) in order to determine the ratios of the drugs in combination yielding the optimal assessment of the intensity of interaction. The best D-optimality criteria was found with the 3 design points: ratio 1:0, ratio 1:1 and ratio 0:1 of drug 1 to drug 2 in terms of potency equivalents ($R = 0$, $R = 0.5$ and $R = 1$). Note that ratios 1:0 and 0:1 are necessary for the estimation of the mean potency of drug 1 and drug 2 when given alone. Also, the maximal departure from the additivity line is observed for $R = 0.5$.

Therefore, for future studies, emphasis should be placed on the replication of fewer design points, more than on a diversity of the design points. Irrespective of the intended method of data analysis, it is clear that the single agents and the ratio 1:1 should be studied more intensively than any of the other combination ratios. However, additional combination points should be added to the experimental design to examine the adequacy of Eq. 5 as the PD model for describing the drug interaction. For instance, such a 3-point design would fail to accurately estimate the intensity of interaction if a given drug combination leads to complex isobol with large departure from the symmetrical parabolic shape predicted by Eq. 5 (e.g., regions of local antagonism and local synergy).

Zhi and Levy suggested that Ccsf are preferable to other data, including doses, for isobolographic analysis involving theophylline (3), because the infused dose and concentrations in serum (plasma) or brain at the pharmacological end-point increased with increasing infusion rate and, therefore, decreasing duration of infusion, whereas CSF concentrations were infusion rate independent (2). We also report on the linearity between Ccsf and dose. Similar findings were obtained with pefloxacin at the Ccsf level (Fig. 3) and agreed with previous data (4). Since pefloxacin and theophylline exhibit linear relationships between Ccsf and doses, it is possible to extrapolate our conclusions on the antagonistic nature of the drug interaction from the dose level to the biophase level.

In general, for compounds exhibiting a linear relationship between plasma (or CSF) concentration and dose (e.g., pefloxacin, theophylline), it is possible to extrapolate the observations made at the dose level to the CSF concentration level. However, if drug concentrations are dependent on the rate and duration of infusion, e.g., norfloxacin (4), a nonlinear relationship is likely to be observed between Ccsf and dose. We can anticipate that for such drugs an extrapolation of the nature and intensity of the drug interaction from the dose level to the biophase level will be hazardous.

Finally, the significant antagonistic nature of the convulsant interaction between pefloxacin and theophylline is notable considering that *in vitro* experiments have previously demonstrated that the combination of another quinolone, ciprofloxacin, with theophylline, was additive in reducing the level of muscimol binding to the $GABA_A$ receptors (15). Since the mechanism by which each compound leads to CNS excitatory effect has not been clearly established, the mechanistic basis for this antagonistic interaction will not be proposed. However, we could anticipate that $GABA_A$ is one of several potential contributors to the drug interaction.

In conclusion, we have demonstrated the suitability of a new isobolographic approach with measurements of the drug concentration in the biophase at the onset of activity, to characterize the nature and the intensity of the *in vivo* pharmacodynamic (convulsant) interaction between two drugs (i.e., pefloxacin and theophylline). This approach was specifically developed for direct assays, in which the measured endpoints are doses of agents that produce a prespecified effect rather than the biological effect itself. The use of direct assays is rare, but is critical for some applications. The mechanistic basis of the antagonistic nature of the interaction between theophylline and pefloxacin is being explored in ongoing studies.

ACKNOWLEDGMENTS

This work was supported in part by the NIH grants RR10742 and CA16056.

REFERENCES

1. R. A. Upton. Pharmacokinetic interactions between theophylline and other medication, Part I. *Clin. Pharmacokinet.* **20**:66–80 (1991).
2. I. M. Ramzan and G. Levy. Kinetics of drug action in disease states. XIV. Pharmacodynamics of theophylline-induced seizures in rats. *J. Pharmacol. Exp. Ther.* **236**:708–713 (1986).
3. J. Zhi and G. Levy. Isobolographic assessment of the convulsant

interaction between theophylline and caffeine or pentylenetetrazol in rats. *J. Pharm. Sci.* **79**:678–681 (1990).
4. A. Delon, F. Huguet, Ph. Courtois, J. M. Vierfond, S. Bouquet, and W. Couet. Pharmacokinetic-pharmacodynamic contributions to the convulsant activity of pefloxacin and norfloxacin in rats. *J. Pharmacol. Exp. Ther.* **280**:983–987 (1997).
5. U. Jaehde, M. W. E Langemeijer, A. G. de Boer, and D. D. Breimer. Cerebrospinal fluid transport and disposition of the quinolones ciprofloxacin and pefloxacin in rats. *J. Pharmacol. Exp. Ther.* **263**:1140–1146 (1992).
6. L. Levasseur, H. Faessel, H. K. Slocum, and W. R. Greco. Precision and pattern in 96-well plate cell growth experiments, *Proc. Biopharm. Sect. Am. Stat. Assoc.* 227–232 (1995).
7. W. R. Greco, G. Bravo, and J. C. Parsons. The search for synergy: a critical review from a response surface perspective. *Pharmacol. Rev.* **47**:331–385 (1995).
8. N. Draper and H. Smith. *Applied Regression Analysis, 2nd edition.* In John Wiley & Sons (eds.), New York, 1981.
9. M. C. Berenbaum. Synergy, additivism and antagonism in immunosuppression. *Clin. Exp. Immunol.* **25**:1–18 (1977).
10. SAS Institute Inc. *SAS/STAT User's Guide*, Version 6, Fourth Edition, Volume 2, SAS Institute Inc., Cary, NC, 1989.
11. M. L. Ralston and R. I. Jennrich. DUD, a derivative-free algorithm for nonlinear least squares. *Technometrics* **20**:7–14 (1978).
12. A. Delon, C. Pariat, Ph. Courtois, S. Bouquet, and W. Couet. A new approach for early assessment of the epileptogenic potential of quinolone. (*submitted*).
13. P. K. Gessner. The isobolographic method applied to drug interaction. In P. L. Morselli, S. Garattini, and S. N. Cohen (eds.), *Drug interactions*, Raven Press, New York, 1974, pp. 349–362.
14. D. Z. D'Argenio and A. Schumitzky. *ADAPT II User's guide: Pharmacokinetic / Pharmacodynamic systems analysis software.* Biomedical Simulations Resource, Los Angeles, CA, 1997.
15. S. Segev, M. Rehavi, and E. Rubinstein. Quinolones, theophylline and diclofenac interactions with the γ-aminobutyric acid receptor. *Antimicrob. Agents Chemother.* **32**:1624–1626 (1988).