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

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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 \(-0.415 \pm 0.069\) and \(-0.567 \pm 0.079\), 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 pentylentetrazol (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 (± 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, Pietremont 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.
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, $s_i^2$, are linearly related to means, $X_i$, 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 $(C_p)$, UF concentrations $(C_u)$ or CSF concentrations $(C_{csf})$, 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, $\phi_i$, is found to be close to 2; the parameter $\phi_2$ 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 $\phi_i$.

$$ s_i^2 = \phi_2 \bar{X}_i^2 $$  

(1)

For each of the drugs, the relationship between CSF 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; $\beta_1$ is the slope of the linear relationship; and $\beta_2$ is a curvature factor. When the parameter estimate of $\beta_2$ is significantly different from zero, a nonlinearity among dose and concentration is indicated.

$$ Y = \beta_1 X + \beta_2 X^2 $$  

(2)

The dose-dependency at the level of the unbound plasma fraction $(f_u)$ and CSF/$f_u$ ratio were studied for each drug. In Eq. 3, $X$ is the dose of pefloxacin (or theophylline); $Y$ is $f_u$, or the CSF/$f_u$ ratio of pefloxacin (or theophylline); $\beta_0$ is the intercept parameter; and $\beta_1$ is the slope of the linear relationship between $Y$ and the dose of pefloxacin (or theophylline). When the parameter estimate of $\beta_1$ is significantly different from zero, a dose-dependence of $f_u$, or CSF/$f_u$, is suggested.

$$ Y = \beta_0 + \beta_1 X $$  

(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 $IC$ 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, $IC$ 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 $IC$ used for this direct assay is a measure of central tendency of the tolerance distribution for the induction of seizures. The interaction parameter is $\alpha$ (7). The absolute magnitude of $\alpha$ is directly related to the degree of bowing of the isobol. When $\alpha$ is positive, Loewe synergy is indicated, whereas a negative value of $\alpha$ reflects Loewe antagonism. The interaction is concluded to be Loewe additive if the 95% confidence interval for $\alpha$ encompasses zero.

$$ \frac{C_2}{IC_2} = \frac{1 - C_1}{IC_1} \frac{1 + \alpha \frac{C_1}{IC_1}}{1 + \alpha \frac{C_2}{IC_2}} $$  

(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**

<table>
<thead>
<tr>
<th>Drug combination</th>
<th>Pefloxacin: theophylline ratio$^a$</th>
<th>Pefloxacin: theophylline ratio$^b$</th>
<th>Number of animals</th>
<th>Body weight (g)</th>
<th>Infusion time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0:0:0</td>
<td>\infty</td>
<td>4</td>
<td>245 ± 7</td>
<td>26.7 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>3.6:0.4</td>
<td>19.8</td>
<td>4</td>
<td>240 ± 13</td>
<td>25.4 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>3.2:0.8</td>
<td>8.8</td>
<td>5</td>
<td>239 ± 15</td>
<td>29.1 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>2.8:1.2</td>
<td>5.1</td>
<td>5</td>
<td>239 ± 11</td>
<td>30.5 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>2.4:1.6</td>
<td>3.3</td>
<td>5</td>
<td>240 ± 10</td>
<td>38.9 ± 4.1</td>
<td></td>
</tr>
<tr>
<td>2.0:2.0</td>
<td>2.2</td>
<td>5</td>
<td>237 ± 10</td>
<td>34.6 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>1.6:2.4</td>
<td>1.5</td>
<td>8</td>
<td>244 ± 6</td>
<td>41.4 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>1.2:2.8</td>
<td>0.9</td>
<td>5</td>
<td>239 ± 4</td>
<td>44.8 ± 5.0</td>
<td></td>
</tr>
<tr>
<td>0.8:3.2</td>
<td>0.6</td>
<td>3</td>
<td>230 ± 11</td>
<td>51.2 ± 5.3</td>
<td></td>
</tr>
<tr>
<td>0.4:3.6</td>
<td>0.2</td>
<td>5</td>
<td>241 ± 7</td>
<td>49.0 ± 4.6</td>
<td></td>
</tr>
<tr>
<td>0.0:4.0</td>
<td>0</td>
<td>5</td>
<td>237 ± 8</td>
<td>48.5 ± 2.3</td>
<td></td>
</tr>
</tbody>
</table>

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.