# Antagonistic Interaction Between the Convulsant Activities of Pefloxacin and Its Main Metabolite Norfloxacin in Rats

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A very elegant approach has been developed several years ago by Danhof and Levy to investigate the kinetics of drug action in disease states (1). It consists of administering the drug intravenously to rats at various rates and measuring drug concentrations in various tissues or biological fluids at the onset of activity in order to characterize the biophase. For drugs acting at the central level, cerebrospinal fluid (CSF) has frequently been found to be part of the biophase.

Because this *in vivo* approach may allow distinction between the pharmacokinetic contribution (ability to reach receptors) and the pharmacodynamic contribution (affinity for these receptors) to the observed effect, it has recently been proposed to compare the convulsant activity of fluoroquinolones (2). These antimicrobial agents are generally well tolerated, but central nervous system side effects may occur, including seizures on rare occasions (3).

Two representative compounds were initially used: pefloxacin as a relatively lipophilic fluoroquinolone with a methyl on the piperazine heterocycle, and norfloxacin as a relatively hydrophilic fluoroquinolone without a methyl on the piperazine heterocycle (2). The approach was then used for an interspecies study (4), and extended to the comparison of a larger number of fluoroquinolones (5). However, among several limitations of the method, the presence of one or several metabolites in the biophase at the onset of activity adds complexity to data interpretation (6). We have recently developed a new quantitative isobolographic approach to characterize the nature and intensity of the interaction between pefloxacin and theophylline, after determination of their concentrations in the biophase at the onset of maximal seizures (7). This approach is now used to characterize the nature of the convulsant interaction between pefloxacin and norfloxacin, its major metabolite in several animal species, including rats (8,9).

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## MATERIALS AND METHODS

### Animals and Surgery

This work was done in accordance with the *Principles of Laboratory Animal Care* (NIH Publication #85-23, revised 1985). Male Sprague Dawley rats (n = 42) from Depres Breeding Laboratories (St Doulchard, France), were housed in the Animal Breeding Facilities of the Laboratory (authorization N°: 0028). Their mean body weight was equal to 250 ± 15 g (mean ± SD). The animals were placed in wire cages in a 12-hour light-dark cycle for one week to adjust to the new environment and to overcome possible stress incurred during transit. They had free access to food (Extralabo M20, Pietrement Laboratories, France) and tap water. Cannulas were implanted in the left jugular vein of the animals the day prior to the experiment, as previously described (2). Animals were then housed individually in plastic cages. Food was withdrawn 12 hours before the experiment, but the animals had free access to water until drug infusion.

### Drug Administrations and Samples Collection

A commercially available solution of pefloxacin methane sulfonate (Bellon Laboratories) titrating 240 mmol/L of pefloxacin, and a solution of norfloxacin hydrochloride (Sigma) in 5% glucose titrating 240 mmol/L, were used for this study. 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 in one, and norfloxacin in the other. Flow rates of each syringe were adjusted in order to achieve the desired rate of drug delivery, with a total flow rate equal to 4 ml/hr (Table I). Animals were kept under a heating lamp to maintain body temperature. The infusion was stopped when the animals exhibited maximal seizures. Drug administration was conducted between 2:00 p.m. and 6:00 p.m. Samples were collected as previously described (2).

### Analytical Assay

Fluoroquinolone concentrations were determined by high performance liquid chromatography as previously described (2). The limit of quantification was 0. 15 μmol/L in plasma, plasma ultrafiltrate (UF) and CSF for the two quinolones. Inter-day coefficients of variation calculated for each compound and estimated by adding known amounts of fluoroquinolones to blank plasma or NaCl 0.9% solution (for UF and CSF) at two concentrations close to the usually measured values, were equal to or less than 8%.

### Data Analysis

A plausible model for the isobol (Eq. I) has been previously derived (10).

$$\frac{C_2}{IC_2} = \frac{1 - \frac{C_1}{IC_1}}{1 + \alpha \frac{C_1}{IC_1}} \qquad (I)$$

In Eq. I, for drug 1 and drug 2, C is the dose, or concentration, of drug in combination required to induce maximal seizures

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**Table I.** Summary of Experimental Conditions of the Interaction Study, Data are Presented as Mean ± SE

| Drug combination | | | | |
|---|---|---|---|---|
| Pefloxacin: norfloxacin ratio[a] | Pefloxacin: norfloxacin ratio[b] | Number of animals | Body weight (g) | Infusion time (min) |
| 4.0:0.0 | — | 8 | 256 ± 7 | 25.6 ± 1.2 |
| 3.6:0.4 | 9.0 | 5 | 246 ± 4 | 28.2 ± 3.8 |
| 3.2:0.8 | 4.0 | 8 | 248 ± 4 | 31.5 ± 2.3 |
| 2.7:1.3 | 2.1 | 5 | 260 ± 3 | 30.1 ± 1.5 |
| 2.0:2.0 | 1.0 | 3 | 250 ± 6 | 33.0 ± 1.7 |
| 1.3:2.7 | 0.5 | 3 | 254 ± 6 | 35.5 ± 1.1 |
| 0.0:4.0 | 0.0 | 10 | 250 ± 5 | 30.0 ± 2.3 |

[a] Ratio of flow rates; the total flow rate was constant at 4.0 ml/hr.
[b] Ratio of input rates in molar units.

in rats, $\overline{IC}$ is the geometric mean dose, or concentration, of drug which when given alone was required to induce maximal seizures, and $\alpha$ is the interaction parameter (11). 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. However in an isobologram, the Y-variable is only indirectly caused by the X-variable, precluding the use of standard regression approaches. In order to arrange for the X-variable to 'cause' the Y-variable in a logical manner, we have recently developed a new modeling approach (Eq. II) based upon a data transformation (7).

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

where

$$R = \frac{\frac{C_1}{\overline{IC_1}}}{\frac{C_1}{\overline{IC_1}} + \frac{C_2}{\overline{IC_2}}} = \frac{\overline{IC_2}}{\overline{IC_2} + \overline{IC_1}\frac{C_2}{C_1}}$$

and

$$CI = \frac{C_1}{\overline{IC_1}} + \frac{C_2}{\overline{IC_2}}$$

In Eq. II, CI is the combination index, R is the proportion of pefloxacin in the mixture expressed in terms of mean drug potency equivalents, and $\alpha$ is the interaction parameter (10). In agreement with the results of an extensive Monte Carlo simulation (7), which demonstrated that biases in the parameters estimates were least when the equation was fitted to data with unweighted rather than weighted nonlinear regression, Eq. II was fit to the transformed data (R;CI) with unweighted non linear regression performed with the procedure NLIN in SAS version 6.11 for Windows (7).

## RESULTS

Under the experimental conditions, the total infused volume ranged between 1.4 and 3.1 mL, and maximal seizures occurred most often within 25 and 35 minutes (Table I). The mean potencies for pefloxacin, $\overline{IC_P}$, and norfloxacin, $\overline{IC_N}$, when each was given alone, are shown in Table II. The ratio of the mean potency of pefloxacin to norfloxacin at the levels of doses, Cp, Cu and Ccsf were 0.835, 0.324, 0.285 and 5.42, respectively. Only CSF concentrations reflect concentrations in the biophase for the two compounds (1). Therefore on the basis of the CSF concentrations, the previously defined intrinsic convulsant activity of norfloxacin was 5.42 fold higher than that of pefloxacin. The much lower ratio value for doses reflect the diminished ability of norfloxacin to diffuse within the CSF, as can be seen from the Ccsf/Cu ratios, respectively equal to 82.8% and 4.36%, for pefloxacin and norfloxacin administered alone. Differences between the intrinsic convulsant activity of the two compounds (pharmacodynamic contribution), and between their ability to diffuse within CSF (pharmacokinetic contribution), compensate almost perfectly between each other, so that their apparent convulsant potencies were significantly different at the CSF level, and virtually identical at the dose level.

Estimates of the interaction parameter $\alpha$, characteristic of the nature and the intensity of the drug interaction are shown in Table II. The interaction was found Loewe antagonistic at the level of Ccsf, Cu and Cp as well as Dose, since $\alpha$ was significantly lower than 0. The intensity of the interaction was similar when assessed at the biophase level or not, in particular

**Table II.** Mean Potency (Geometric Average) of Pefloxacin ($\overline{IC_P}$) and Norfloxacin ($\overline{IC_N}$) Estimated at the Levels of Doses, Plasma, UF, and CSF in the Present Study, at the Level of CSF from 3 Previous Studies Separately, and Pooled to get the Geometric Mean Potencies from These 4 Separate Studies.

| | $\overline{IC_P}$ | $\overline{IC_N}$ | $\alpha$ |
|---|---|---|---|
| Dose (μmol/kg) | 1590 [1451–1742] (10) | 1904 [1691–2144] (8) | −0.615 ± 0.068 [−0.753; −0.477] |
| Plasma (μmol/L) | 601 [561–644] (10) | 1854 [1555–2209] (8) | −0.572 ± 0.18 [−0.939; −0.205] |
| UF (μmol/L) | 378 [350–408] (10) | 1326 [1097–1603] (8) | −0.649 ± 0.16 [−0.969; −0.329] |
| CSF (μmol/L) | 313 [292–337] (10) | 57.8 [47.5–70.4] (8) | −0.766 ± 0.062 [−0.890; −0.642] |
| CSF (μmol/L) (Ref 2) | 379 [369–389] (28) | 46.7 [42.8–50.6] (25) | |
| CSF (μmol/L) (Ref 4) | 323 [308–338] (6) | 48.3 [44.2–52.4] (8) | |
| CSF (μmol/L) (Ref 5) | 313 [288–339] (8) | 58.9 [49.3–67.1] (8) | |
| CSF (μmol/L) (Pooled from 4 separate studies) | 348 [336–360] (52) | 50.4 [46.9–53.9] (49) | |

*Note:* Values are followed by the 95% confidence interval into brackets and the number of determinations into parentheses. The estimates (± SE) of the interaction parameter $\alpha$ from the present study are also presented.

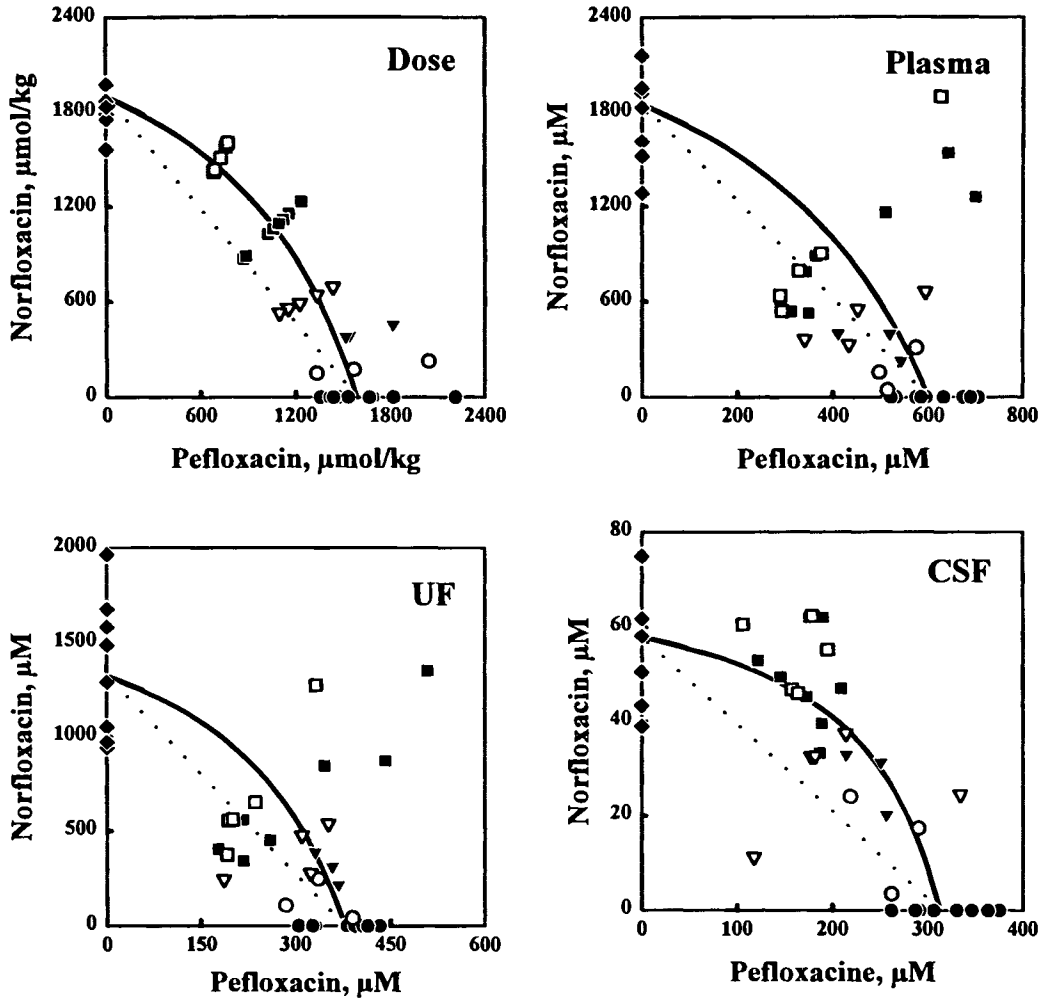


**Fig. 1.** Isobolographic representation of the raw data for the injected dose and the corresponding plasma, UF and CSF concentrations. The dotted line is the theoretical additivity isobol. The thick solid line from Figure III was transformed back to the isobologram coordinates. Different symbols indicate each absolute ratio of pefloxacin dose to norfloxacin dose in the mixture.

at the dose level, except for lower variability observed in the biophase (Fig. 1).

## DISCUSSION

One of the basic requirements for the recently proposed quantitative analysis of isobolographic data (10) used in this study is that $\overline{IC}_P$ and $\overline{IC}_N$ estimates must be close to the true values. It was therefore interesting to compare the various estimates obtained throughout three previous studies and the present one (Table II). Only CSF concentrations are compared, since other values may vary with the experimental conditions, in particular the duration of infusion (1,2). These data show the inter-occasion variability is relatively limited. Individual potency measurements collected within three previously published and independent studies (2,3,5), and during the present one, were pooled in order to estimate the following overall geometric mean potencies: $\overline{IC}_P$ = 348 µmol/L (n = 52) and $\overline{IC}_N$ = 50.4 µmol/L (n = 49) (Table II), indicating that the intrinsic convulsant activity of norfloxacin is 6.9 fold higher on average than that of pefloxacin.

The approach used in this study does not require any mechanistic assumption, one should recall that convulsions occur when concentrations of each drug reach a certain level in the biophase. Since Eq. II has been developed to characterize the pharmacodynamic interaction between the two compounds, it applies primarily to concentrations within the biophase, which constitutes the driving force of the system. However when Eq. II was applied to CSF, Cp, Cu and Doses data, the same general shape was observed (Fig. 1), and almost similar estimates of $\alpha$ were obtained (Table II). Therefore in this particular situation, Doses, Cp and Cu are as good as Ccsf to characterize the interaction, except for the higher variability.

As previously noted (5), the tonic phase easily observable with pefloxacin was absent on most occasions with norfloxacin, indicating that not only quantitative differences exist between the convulsive activities of these two compounds. Qualitative

differences may be due to the involvement of various types of mediators in the initiation or/and maintenance seizures, which could then be responsible for the antagonistic interaction between the two compounds. However our approach to characterize the nature and the intensity of the drug interaction is based on an empirical combination index model derived from the non-mechanistic-based theory of additivity by Loewe (12) and concepts of interaction from Berenbaum (13). Therefore the mechanistic basis for this antagonistic interaction is still unknown, but this question is being explored in ongoing studies.

The quantitative approach used in this study has been recently developed by our group in order to interpret data from the combination of pefloxacin plus theophylline (7). With existing methods that are based on the visual inspection of an isobologram, decisions regarding the nature of the drug interaction in a direct assay are made by looking at the respective position of the experimental points and the additivity line (14). However the Combination Index method yields the estimation of an interaction parameter, which characterizes not only the nature of the drug interaction, but also its intensity. This method enables direct comparison of the degree of interaction across studies. For example, the interaction at the CSF level between pefloxacin and theophylline was less antagonistic, $\alpha = -0.415 \pm 0.069$ (7), than that of pefloxacin and its metabolite norfloxacin, $\alpha = -0.766 \pm 0.062$.

It was possible to conduct the pefloxacin-norfloxacin interaction study using the isobolographic approach because we have previously shown that concentrations of the metabolite norfloxacin in the biophase at the onset of maximal effect can be neglected following intravenous infusion of the parent drug pefloxacin (2), making possible assessment of $\overline{IC}_P$ and $\overline{IC}_N$. Such would not be the case with diazepam, since metabolites formation and diffusion within CSF are much more rapid, leading to relatively high concentrations of these metabolites in the biophase at the onset of activity. Therefore the approach used by Klockowski and Levy, to characterize the hypnotic activity of diazepam alone, consisting of subtracting the effect of each metabolite previously assessed after direct administration, was probably the only possibility (6). However this approach assumes implicitly that the pharmacodynamic interactions between the parent drug and its metabolites is additive, which may not be true as demonstrated for the pefloxacin-norfloxacin convulsant interaction.

In conclusion this study is a good although still unexplained example of a Loewe antagonistic interaction between the undesirable side effects of a parent drug and its main metabolite *in vivo*.

## REFERENCES


1. M. Danhof and G. Levy. Kinetics of drug action in disease states. I. Effect of infusion rate on phenobarbital concentrations in serum, brain and cerebrospinal fluid of normal rats at onset of loss of righting reflex. *J. Pharmacol. Exp. Ther.* **229**:44–50 (1984).
2. A. Delon, F. Huguet, P. 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).
3. W. Christ. Central nervous system toxicity of quinolones: human and animal findings. *J. Antimicrob. Chemother.* **26 (Suppl.B)**:219–225 (1990).
4. A. Delon, C. Pariat, P. Courtois, S. Bouquet, and W. Couet. A new approach for early assessment of the epileptogenic potential of quinolones. *Antimicrob. Agents Chemother.* **42**:2756–2758 (1998).
5. A. Delon, S. Bouquet, F. Huguet, V. Brunet, P. Courtois, and W. Couet. Pharmacokinetic-Pharmacodynamic contributions to the convulsant activity of fluoroquinolones in rats. *Antimicrob. Agents Chemother.* **43**:1511–1515 (1999).
6. P. M. Klockowski and G. Levy. Kinetics of drug action in disease states. XXIV. Pharmacodynamics of diazepam and its active metabolites in rats. *J. Pharmacol. Exp. Ther.* **244**:912–918 (1987).
7. L. M. Levasseur, A. Delon, W. R. Greco, P. Faury, S. Bouquet, and W. Couet. Development of a new quantitative approach for the isobolographic assessment of the convulsant interaction between pefloxacin and theophylline in rats. *Pharm. Res.* **15**:1069–1076 (1998).
8. G. Montay, Y. Goueffon, and F. Roquet. Absorption, distribution, metabolic fate, and elimination of pefloxacin mesylate in mice, rats, dogs, monkey and humans. *Antimicrob. Agents Chemother.* **25**:463–472 (1984).
9. 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).
10. 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).
11. W. R. Greco, H. S. Park, and Y. M. Rustum. Application of a new approach for the quantitation of drug synergism to the combination of cis-diamminedichloroplatinum and 1-beta-D-arabinofuranosylcytosine. *Cancer Res.* **50**(17):5318–27 (1990).
12. S. Loewe and H. Muischnek. Effect of combinations: mathematical basis of problem. *Arch. Exp. Pathol. Pharmakol.* **114**:313–326 (1926).
13. M. C. Berenbaum. The expected effect of a combination of agents: the general solution. *J. Theor. Biol.* **114**:413–431 (1985).
14. 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).