Pharmacokinetic-pharmacodynamic modelling of the convulsant interaction between norfloxacin and biphenyl acetic acid in rats

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1 Fluoroquinolones (FQs) are associated with a low incidence of central nervous system (CNS) side effects, possibly leading to convulsions, especially when co-administered with nonsteroidal anti-inflammatory drugs (NSAIDS). Although the *in vivo* pro-convulsant activity of NSAIDS is essentially unknown, the convulsant potential of FQs is traditionally evaluated by *in vitro* γ-aminobutyric acid (GABA) binding experiments in the presence of 4-biphenyl acetic acid (BPAA), the active metabolite of fenbufen.

2 The aim of this study was therefore to investigate the BPAA-norfloxacin convulsant interaction *in vivo*.

3 Male Sprague-Dawley rats ($n = 27$) were given BPAA orally, at various doses 1 h before norfloxacin infusion, which was maintained until the onset of maximal seizures, when cerebrospinal fluid (CSF) and plasma samples were collected for analysis.

4 An inhibitory E_{max} effect model with a baseline effect parameter was fitted to the norfloxacin versus BPAA concentrations in the CSF, previously shown to be part of the biophase. This model includes three parameters: the concentrations of norfloxacin in the absence of BPAA ($C_{CSF0, Nor}$), and when BPAA concentration tends toward infinity ($C_{CSFbase, Nor}$), and the BPAA concentration for which half of the maximal effect is observed ($C_{CSF50, BPAA}$). The maximal proconvulsant effect of BPAA is given by the $C_{CSF0, Nor} / C_{CSFbase, Nor}$ ratio, estimated to approximately 6 in this study.

5 Derived models were developed in plasma to account for the non-linear CSF diffusion of norfloxacin and protein binding of BPAA.

6 In conclusion this study has shown that the convulsant interaction between norfloxacin and BPAA in rats, can be adequately characterized by modelling of the CSF concentrations of the two drugs at the onset of activity, following their administration in various proportions.

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Abbreviations: BPAA, 4-biphenyl acetic acid; CNS, central nervous system; CSF, cerebrospinal fluid; FQs, fluoroquinolones; GABA, γ-aminobutyric acid; h.p.l.c., high performance liquid chromatography, NSAIDs, nonsteroidal anti-inflammatory drugs

Introduction

Fluoroquinolone (FQ) antimicrobial agents are a class of inhibitors of bacterial topoisomerases. Initially, FQs have been used in the treatment of urinary infections, but because of their activity against Gram-negative bacteria and also their potency against Gram-positive bacteria, the new FQs are used in a variety of bacterial infections such as sexually transmitted diseases, gastrointestinal or respiratory tract infections, as well as skin and bones infections (Walker & Wright, 1991; Moellering, 1996; Hopper, 1998). Because FQs can diffuse into the CNS, they have been occasionally proposed as an alternative in the treatment of CNS infections (Scheld, 1989; Hasbun & Quagliarello, 1998). They are generally well tolerated but CNS disorders including headache, confusion, hallucination, anxiety, nervousness, nightmares (Anastasio, 1988; Christ, 1990) have been reported in ~2% of patients (Rodvold & Piscitelli, 1993). The incidence of severe CNS side effects is quite low, but seizures have been more frequently observed in patients receiving FQs in combination with NSAIDs such as fenbufen (Simpson & Brodie, 1985; Arcieri *et al.*, 1987; Anastasio *et al.*, 1988). It is usually admitted that the central excitatory effect of FQs results from an inhibition GABA$_A$

binding to its receptors (Tsuji *et al.*, 1988; Akahane *et al.*, 1989; Tsutomi *et al.*, 1994). However *in vitro* GABA binding experiments have demonstrated that FQs are weak GABA antagonists (Segev *et al.*, 1988; Akahane *et al.*, 1989; Hori & Shimada, 1993), except in very specific situations such as in the presence of BPAA, the active metabolite of fenbufen (Halliwell *et al.*, 1993). Therefore predicting the convulsant risk associated to FQs administration in patients, from *in vitro* GABA binding experiments in the presence of BPAA, as most often done and accepted by registration agencies (Akahane *et al.*, 1989; Hori & Shimada, 1993; Tsutomi *et al.*, 1994; Akahane *et al.*, 1994), may not be appropriate, especially because BPAA is never associated to FQs in clinical practice. In fact we have recently demonstrated some agreement but also inconsistencies, between the convulsant risk predicted from *in vitro* GABA binding experiments in the presence of BPAA, and the convulsant activity actually observed *in vivo* in the absence of BPAA (Delon *et al.*, 1999a). In order to elucidate these discrepancies, experiments should be conducted *in vivo* to compare the convulsant activities of various FQs in the absence and in the presence of BPAA. However such investigations are likely to be complicated by various phenomenon including equilibration delay (Delon *et al.*, 1997), saturable central diffusion of the FQ (Jaehde *et al.*, 1992) as well as non-linear protein binding of BPAA, and results are likely to vary with the

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observation site or/and the relative concentration of each compound. It seems therefore necessary to start with a detailed investigation of the interaction between a selected FQ such as norfloxacin and BPAA, using an experimental approach allowing distinction between the pharmacokinetic and pharmacodynamic contributions to the overall effect, after administering the two compounds at various doses, in order to describe the whole phenomenon as previously done (Levasseur *et al.*, 1998; Delon *et al.*, 1999b).

Methods

Animals

This work was done in accordance with the Principles of Laboratory Animal Care (NIH Publication #85-23, revised 1985), and the study protocol was approved by the local ethic committee. Male Sprague Dawley rats ($n = 27$) from Depres Breeding Laboratories (St Doulchard, France), were housed in the Animal Breeding Facilities of the Laboratory (authorization No: 0028). Their mean body weight was equal to 254 ± 16 g. The animals were placed in wire cages in a 12 h light-dark cycle for 5 days before the beginning of experiment to adjust to the new environment. During this period, they had free access to food (Extralabo M20, U.A.R. Laboratories, France) and water.

Surgery

A polyethylene cannula (0.58 mm inside, 0.96 mm outside diameter, Plastimed Laboratories, France) was implanted in the right jugular vein of the animals the day prior to the experiment under a 60 mg kg^{-1} sodium pentotal (Sanofi Laboratories, France) intraperitoneal anaesthesia. Following the surgery, rats were kept under a heating lamp. After first signs of movement, the animals were placed into individual plastic cages. Food was withdrawn 12 h before the experiment, but the animals had free access to water until drug infusion.

Solutions for administration

The BPAA suspension was prepared for three different doses (10, 30, 50 mg kg^{-1} corresponding to 47, 141 and 236 μmol kg^{-1}) by a mixture of BPAA (batch 125H3426, Sigma, France) in a sodium carboxymethyl cellulose (batch F17493, French Pharmaceutics Cooperation, France) 0.5% (w v^{-1}) (Tsutomi *et al.*, 1994). A 240 mM solution of norfloxacin hydrochloride dissolved in 5% glucose at pH 5.5, was used for intravenous administration (Delon *et al.*, 1997).

Drugs administration

The day after surgery, the BPAA suspension was given orally by gastric tubing 1 h before the beginning of norfloxacin infusion. Three different groups were performed on the basis of the three doses of BPAA administered ($n = 5 - 8$ per group). A solution of sodium carboxymethyl cellulose 0.5% devoid of BPAA was administered orally for the control group ($n = 8$).

For norfloxacin administration, the jugular vein cannula was connected to a motor-driven syringe pump (SE400B, Vial Medical, France) containing the norfloxacin solution, at a flow rate of 960 μmol h^{-1}. 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. Drug administration was conducted between 1400 h and 1900 h.

Samples collection

Immediately after exhibiting maximal seizures, rats were anaesthetized with an intramuscular injection of 12.5 mg of ketamin (KETALAR$^®$, 50 mg ml^{-1}, Parke Davis Laboratories, France) and 5 mg of xylazin hydrochloride (ROMPUN$^®$, Bayer Laboratories, France), unless they had died following seizures. In any case CSF was collected within 3 min, as previously described (Anastasio *et al.*, 1988; Delon *et al.*, 1999a). Blood was subsequently withdrawn from the heart, collected in heparinized tubes (VACUTAINER$^®$, Becton Dickinson, France) and immediately centrifuged at 3000 r.p.m. for 10 min (GR 412 model, Jouan, France). Plasma was transferred into two separate tubes. One fraction was kept frozen at $-20°C$ until assayed. The other fraction was ultrafiltered with a Centrifree system (CF50A model, Amicon, France) for determination of unbound concentrations.

Drug analysis

Norfloxacin and BPAA concentrations were determined by h.p.l.c. The norfloxacin and BPAA assays were performed with a Kromasil C18 column (5 μm, 150×3 mm i.d.). The chromatographic system consisted of a Waters 510 model pump and a Gilson 231 autosampler connected to a Kratos 980 fluorimetric detector (excitation wavelength = 280 nm, emission wavelength = 445 nm) for norfloxacin and to a Waters 484 u.v. detector at the wavelength of 254 nm for the BPAA. Data were recorded and processed using a Waters 746 integrator.

For norfloxacin assay, the mobile phase consisted of 0.1 M aqueous citric acid solution containing 8% (v v^{-1}) acetonitrile and 10 mM tetra butyl ammonium perchlorate, and the flow rate was 0.8 ml min^{-1}. Norfloxacin was assayed in CSF and UF by direct injection after appropriate dilution (1/10 and 1/40 respectively) in a mixture of 0.1 M citrate buffer (pH = 3). Plasma samples were diluted appropriately by addition of a 1.7% (v v^{-1}) perchloric acid. The mixture was then centrifuged (3000 r.p.m., 10 min, 5°C) and 20 μl of the supernatant was injected onto the column (Delon *et al.*, 1997).

For BPAA determination, the mobile phase consisted of a mixture of 50% (v v^{-1}) acetonitrile, 49% (v v^{-1}) water (Milli-Q) and 1% (v v^{-1}) acetic acid, the flow rate was 0.8 ml min^{-1}. BPAA was assayed in CSF and UF by direct injection after appropriate dilution (1/10) in methanol. Plasma samples were diluted appropriately by addition of a 1.7% (v v^{-1}) perchloric acid. The mixture was then centrifuged (3000 r.p.m., 10 min, 5°C) and 20 μl of the supernatant was injected onto the column.

Theoretical analysis

Because the convulsant activity of norfloxacin is strictly related to its CSF concentration but not necessarily to its plasma concentration (Delon *et al.*, 1997), the pharmacodynamic interaction between the two drugs was first investigated at the CSF (biophase) level, which was considered as the driving force of the system (Figure 1).

CSF concentrations modelling   An inhibitory E_{max} effect model with a baseline effect parameter (Gabrielsson & Weiner, 1997), was fitted to the CSF norfloxacin versus BPAA

concentrations ($C_{CSF, Nor}$ VS $C_{CSF, BPAA}$). The general form of this equation is as follows:

$$C_{CSF, Nor} = C_{CSF0, Nor} - (C_{CSF0, Nor} - C_{CSF, base, Nor})$$
$$\times \frac{C_{CSF, BPAA}}{(C_{CSF, BPAA} + C_{CSF50, BPAA})} \quad (1)$$

where $C_{CSF0, Nor}$ is the concentration of norfloxacin at the onset of maximal seizures in the absence of BPAA, $C_{CSF, base, Nor}$ is the asymptotic value of norfloxacin concentration when BPAA concentration (C_{BPAA}) tends toward infinity, and $C_{CSF50, BPAA}$ is the concentration of BPAA corresponding to a norfloxacin concentration (C_{Nor}) equal to $\frac{1}{2} \times (C_{O, Nor} - C_{base, Nor})$.

Unbound plasma concentrations modelling Equation 1 was also fitted to the norfloxacin versus BPAA unbound plasma concentrations ($C_{U, Nor}$ vs $C_{U, BPAA}$). However this inhibitory E_{max} effect model with a baseline parameter can adequately describe both sets of data (CSF and free plasma concentrations) only if the CSF diffusion of both compounds is linear. But in these experimental conditions the CSF diffusion of norfloxacin was non-linear. The relationship between CSF and unbound plasma concentrations of norfloxacin was as follows:

$$C_{CSF, Nor} = \frac{C_{CSF_{max}, Nor} \times C_{U, Nor}}{(C_{U50, Nor} + C_{U, Nor})} \quad (2)$$

where $C_{CSF_{max}, Nor}$ is the maximum concentration of norfloxacin achievable into the CSF and $C_{U50, Nor}$ is the unbound norfloxacin concentration that corresponds to $\frac{1}{2}$ of $C_{CSF_{max}, Nor}$.

For BPAA, the relationship between CSF and unbound plasma concentrations was linear.

$$C_{CSF, BPAA} = K_d \times C_{U, BPAA} \quad (3)$$

where K_d is the distribution coefficient of BPAA between CSF and unbound plasma concentrations.

A new relationship (see Equation 4) could then be obtained by integrating Equations 1 and 3 into Equation 2 after reorganization.

This new equation characterizes the indirect relationship between unbound plasma concentrations of norfloxacin and BPAA, and reflects both the pharmacodynamic interaction between the two drugs, and their CSF diffusion characteristics (Figure 1).

Total plasma concentrations modelling Equation 1 was tested again, although from the previous it was not likely to provide good data fitting. The model previously used to characterize the interaction at the unbound concentrations level (Equation 4), may describe the norfloxacin versus BPAA total plasma concentrations ($C_{P, Nor}$, vs $C_{P, BPAA}$), only if plasma protein binding of the two drugs is linear. However plasma protein binding of norfloxacin was linear (and actually almost negligible) but not that of BPAA.

The Langmuir equation (Behm & Wagner, 1981; Benincosa & Morris, 1993; Kochak *et al.*, 1993) (Equation 5a), was successful in fitting BPAA bound (C_B) versus free plasma (C_U) concentrations:

$$C_{U, Nor} = \frac{[C_{U50, Nor} \times (C_{CSF0, Nor} - C_{CSF0, Nor} - C_{CSF base, Nor}) \times \frac{K_d \times C_{U, BPAA}}{[K_d \times C_{U, BPAA}) + C_{CSF50, BPAA}]}]}{C_{CSF_{max}, Nor} - [C_{CSF0, Nor} - (C_{CSF0, Nor} - C_{CSF base, Nor}) \times \frac{K_d \times C_{U, BPAA}}{[(K_d \times C_{U, BPAA}) + C_{CSF50, BPAA}]}]} \quad (4)$$
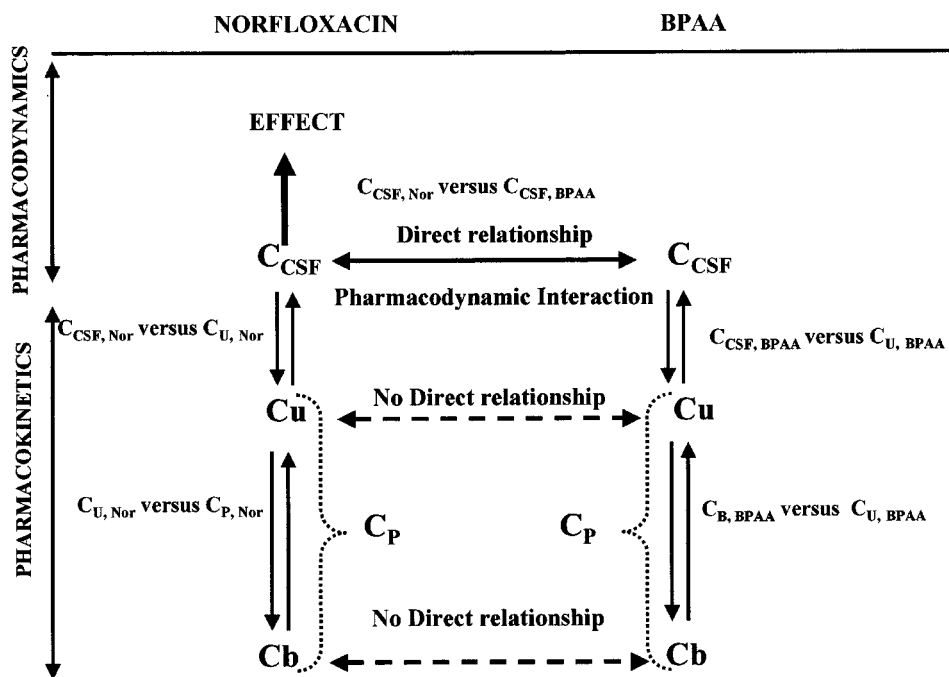

Figure 1 Schematic representation of the convulsant interaction between norfloxacin and BPAA with distinction between the pharmacodynamic interaction of the two compounds in CSF (biophase) and the pharmacokinetic relationships characteristic of the CSF diffusion and plasma protein binding of each compound.

$$C_{B,\,BPAA} = \frac{C_{B_{max},\,BPAA} \times C_{U,\,BPAA}}{(C_{U50,\,BPAA} + C_{U,\,BPAA})} \tag{5a}$$

where $C_{B_{max},\,BPAA}$ is the maximal concentration of BPAA necessary to saturate 100% of binding sites, and $C_{U50,\,BPAA}$ is the unbound BPAA fraction which could saturate 50% of binding sites of BPAA.

And since

$$C_{P,\,BPAA} = C_{U,\,BPAA} + C_{B,\,BPAA}$$

then,

$$C_{P,\,BPAA} = C_{U,\,BPAA} + \frac{C_{B_{max},\,BPAA} \times C_{U,\,BPAA}}{(C_{U50,\,BPAA} + C_{U,\,BPAA})} \tag{5b}$$

By re-organization of the Equation 5b, one obtains unbound concentrations ($C_{U,\,BPAA}$) as a function of the total plasma concentration of BPAA ($C_{P,\,BPAA}$) (see equation 5c)

$$C_{U,\,BPAA} = C_{P,\,BPAA} - C_{B_{max},\,BPAA} - C_{U50,\,BAA} + A \tag{5c}$$

where,

$$A = \frac{\sqrt{(C_{B_{max},\,BPAA} + C_{U50,\,BPAA} - C_{P,\,BPAA})^2 + (4 \times C_{P,\,BPAA} \times C_{U50,\,BPAA})}}{2}$$

Norfloxacin unbound versus plasma total concentrations were linearly related and fitted according to the following equation (Equation 6):

$$C_{U,\,Nor} = fu \times C_{P,\,Nor} \tag{6}$$

where fu is the free fraction of norfloxacin in plasma.

By integrating these plasma protein binding characteristics (Equations 5c and 6) in Equation 4, a new equation could be obtained (see Equation 7), which describes the indirect relationship between total plasma concentrations of norfloxacin and BPAA, and reflects the pharmacodynamic interaction between these two compounds as well as their CSF diffusion and plasma protein binding characteristics. (See Equation 7.)

Modelling and simulations were performed with WinNonlin, version 1.0 (Scientific Consulting, Inc.). Modelling was conducted with uniform weighting. Discrimination between linear and non-linear models of CSF diffusion and protein binding was assessed from various criteria, including visual inspection, residual analysis, sum of squared residuals (SSR), correlation coefficient between observed and predicted values, and Akaïke information criteria (AIC). Data were compared by the non-parametric Kruskal-Wallis test followed by the Dunn's multiple comparison post test, with a level of significance set at $P < 0.05$.

Results

As a result of the interaction between norfloxacin and BPAA, the time of maximal seizures was significantly reduced from 25.6 ± 2.3 min when norfloxacin was infused alone, to 5.1 ± 1.4 min when it was co-administered with the highest oral dose of BPAA equal to 50 mg kg^{-1} (Table 1). Complementary experiments showed that no convulsions appeared after BPAA was administered alone even at an oral dose of 300 mg kg^{-1} (data not shown).

The inhibitory E_{max} effect model with a baseline effect parameter (Equation 1) was appropriate to describe the interaction between BPAA and norfloxacin in the CSF (Figure 2). The mean CSF concentration of norfloxacin at the onset of activity when given alone, was estimated by the modelling procedure ($C_{CSF0,\,Nor}$) to 47.3 ± 2.3 μM (CV = 4.95%), which is virtually identical to the algebraic mean of individual concentrations (47.1 ± 10.7 μM), and in agreement with results obtained from previous experiments (Delon *et al.*, 1997; 1999a,b). The absence of intercept with the abscissa, characteristic of the model, is consistent with the lack of convulsant activity of BPAA administered alone, and is indicative of the existence of a maximum proconvulsant effect. The asymptotic value of norfloxacin concentration when that of BPAA tends toward infinity ($C_{CSFbase,\,Nor}$) was estimated to 7.69 ± 2.23 μM (CV = 29%). The ratio between $C_{CSF0,\,Nor}$ and $C_{CSFbase,\,Nor}$ indicates that BPAA increases the convulsant activity of norfloxacin by approximately 6 fold at the most. Half of the maximum pro-convulsant effect was observed at a CSF concentration of BPAA ($C_{CSF50,\,BPAA}$) equal to 0.80 ± 0.31 μM. This last parameter however was not estimated with a great precision (CV = 39%).

This inhibitory E_{max} effect model did not result in a totally satisfactory fitting of the norfloxacin versus BPAA unbound plasma concentrations. The mean unbound plasma concentration of norfloxacin at the onset of activity when given alone, was correctly estimated by the modelling ($C_{U0,\,Nor} = 1528 \pm 56$,

Table 1 Summary of experimental conditions, data are presented as mean $\pm$ s.d.

BPAA doses	Number of animals	Body weight (g)	Time of convulsions[a] (min)
Controls	8	249 ± 21	25.6 ± 2.3
10 mg kg^{-1} (47 μmol kg^{-1})	5	255 ± 8	24.0 ± 5.3
30 mg kg^{-1} (141 μmol kg^{-1})	6	264 ± 16	6.5 ± 2.8[b]
50 mg kg^{-1} (236 μmol kg^{-1})	8	250 ± 13	5.1 ± 1.4[b]

[a]Statistically different by the Kruskal-Wallis test. [b]Statistically different from controls by the Dunn's test.

$$C_{P,\,Nor} = \frac{1}{fu} \times \frac{C_{U50,\,Nor} \times [C_{CSF0,\,Nor} - (C_{CSF0,\,Nor} - C_{CSF\,base,\,Nor}) \times \frac{K_d \times B}{[(K_d \times A) + C_{CSF50,\,BPAA}]}}{C_{CSF_{max},\,Nor} - [C_{SCF0,\,Nor} - (C_{SCF0,\,Nor} - C_{CSF\,base,\,Nor}) \times \frac{Kd \times B}{[(K_d \times B) + C_{CSF50,\,BPAA}]}} \tag{7}$$

$$\text{where, } B = C_{P,\,BPAA} - C_{B_{max},\,BPAA,\,BPAA} + A$$

CV = 3.7%), but not that of $C_{Ubase, Nor}$ (83.3 ± 59.1 μM, CV = 71%). The ratio between these two estimates would suggest that BPAA may increase the convulsant activity of norfloxacin by up to 18 fold. Furthermore the CSF over unbound norfloxacin concentrations ratio in the absence of BPAA ($C_{CSF0, Nor}$ / $C_{U0, Nor}$) was equal to 3.0%, when the same ratio at lower concentrations of norfloxacin ($C_{CSFbase, Nor}$ / $C_{Ubase, Nor}$) would be equal to 9.2%. This difference suggests saturable CSF diffusion of norfloxacin. In agreement with that observation, a non-linear model (Equation 2) provided better data fitting of the CSF versus unbound norfloxacin concentrations than a linear model, and lead to an estimate of a maximum achievable CSF concentration ($C_{CSF_{max}, Nor}$) equal to 78 ± 13 μM with a corresponding $C_{U50, Nor}$ value equal to 1052 ± 358 μM (Figure 3a). In contrast the CSF diffusion of BPAA was linear (Equation 3) with a coefficient of diffusion close to unity ($K_d = 0.944 ± 0.015$) (Figure 3b). The indirect relationship between the unbound plasma concentrations of the two drugs was well described by Equation 4, using parameters values estimated from the three direct relationships: Equation 1 ($C_{CSF0, Nor}$; $C_{CSFbase, Nor}$; $C_{CSF50, BPAA}$), Equation 2 ($C_{CSF_{max}, Nor}$; $C_{U50, Nor}$) and Equation 3 (K_d). In these conditions, experimental data points were almost exactly superimposed to simulated concentrations (Figure 5a).
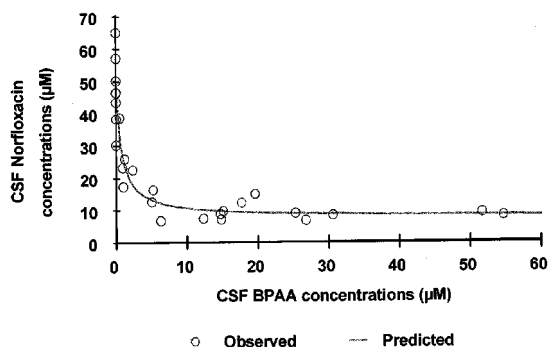

Figure 2 Norfloxacin versus BPAA CSF concentrations at the onset of maximal seizures. Each point represents CSF data from one rat ($n = 27$) obtained following IV infusion of a solution of norfloxacin hydrochloride 240 mM, 1 h after oral administration of BPAA at one of the following doses: 10, 30 or 50 mg kg^{-1}. The control group was obtained after p.o. administration of a solution of carboxymethyl-cellulose 0.5% devoid of BPAA. Norfloxacin versus BPAA CSF concentrations were fitted according to an E_{max} effect model with a baseline effect parameter (Equation 1, solid line), the three parameters estimates were: $C_{CSF0, Nor} = 47.3 ± 2.3$ μM, $C_{CSFbase, Nor} = 7.69 ± 2.23$ μM, $C_{CSF50, BPAA} = 0.80 ± 0.31$ μM.
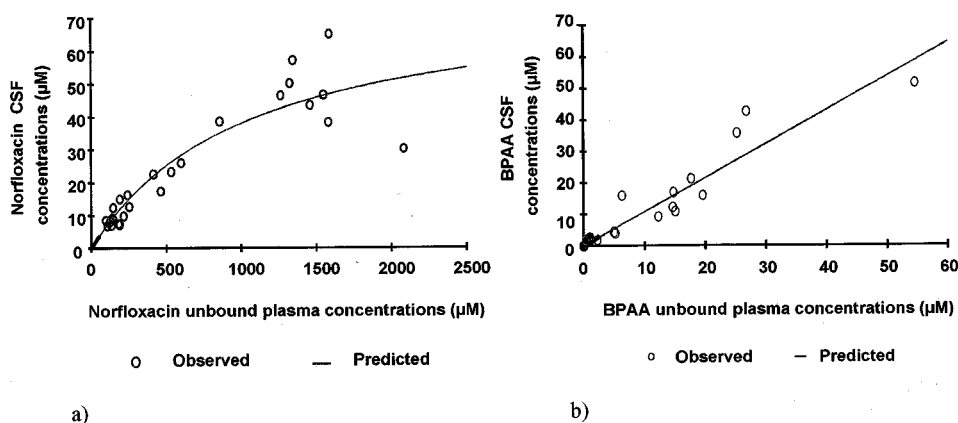

a)

b)

Figure 3 Norfloxacin (a) and BPAA (b) CSF versus unbound plasma concentrations. Each point represents data from one rat ($n = 27$). (a) Pairs of data were fitted according to a non-linear model (Equation 2, solid line), the two parameters $C_{CSF_{max}, Nor}$ and $C_{U50, Nor}$ were respectively estimated to 78 ± 13 μM and 1052 ± 358 μM. (b) Pairs of data were fitted according to a linear model (Equation 3, solid line), with a coefficient of diffusion of BPAA (K_d) equal to 0.944 ± 0.015.
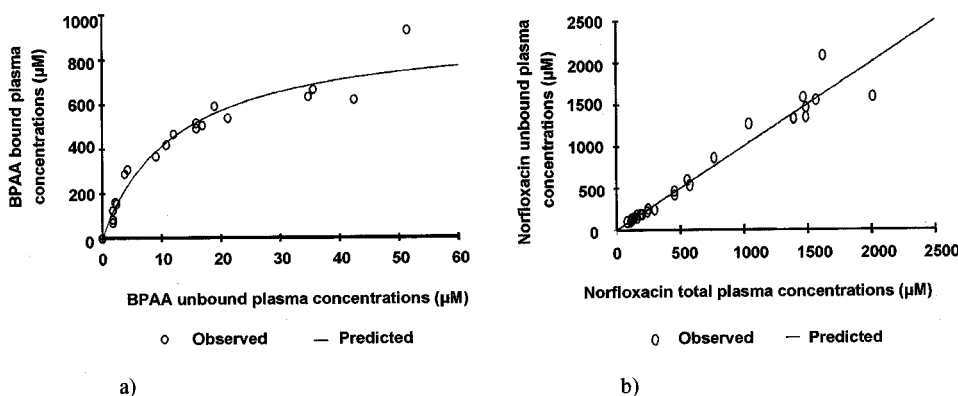

a)

b)

Figure 4 BPAA (a) and norfloxacin (b) plasma protein binding. Each point represents data from one rat ($n = 27$). (a) Plasma protein binding of BPAA followed a non linear model and pairs of data were fitted according to a Langmuir equation (Equation 5a, solid line) with parameters estimates equal to 932 ± 62 μM ($C_{B_{max}, BPAA}$) and 12.7 ± 2.1 μM ($C_{U50, BPAA}$). (b) Pairs of data were fitted according to a linear model (Equation 6, solid line) with the free fraction of norfloxacin (fu) estimated to 0.99 ± 0.03.
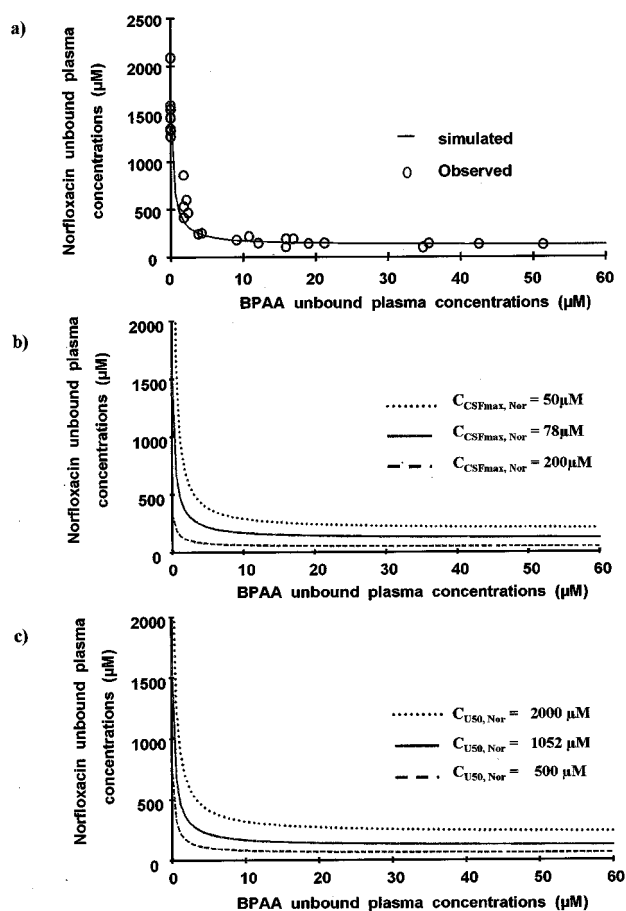
Figure 5 Norfloxacin versus BPAA concentrations in UF. (a) represents the superimposition of the simulated curve from Equation 4 and experimental data points. Norfloxacin versus BPAA unbound plasma concentrations were simulated for various values of the parameters characteristic of the non-linear CSF diffusion of norfloxacin: $C_{CSF_{max}, Nor}$ from 50 μM (top, right) to 200 μM (bottom, left) (b) and $C_{U50, Nor}$ from 500 μM (bottom, left) to 2000 μM (top, right) (c).

The initial E_{max} effect model (Equation 1) did not provide adequate fitting of the total plasma concentrations sets of data at all. In particular a very poor estimate of $C_{Pbase, Nor}$ was obtained (50 ± 76 μM, CV = 154%), with a ratio between $C_{P0, Nor}$ and $C_{Pbase, Nor}$ suggesting a pro-convulsant effect by up to 30 fold. BPAA plasma protein binding was extensive (>96%) and non-linear, with parameters estimated from the Langmuir equation (equation 5a) equal to 932 ± 62 μM ($C_{Bmax, BPAA}$) and 12.7 ± 2.1 μM ($C_{U50, BPAA}$) (Figure 4a). Plasma protein binding of norfloxacin was linear (equation 6) and virtually negligible with an unbound fraction fu = 0.99 ± 0.03 estimated from the slope of the regression (Figure 4b). The indirect relationship between the total plasma concentrations of the two drugs was relatively well described by Equation 7, with parameters values estimated from three corresponding direct relationships (Equations 4, 5a, 6), as illustrated on Figure 6a.

Discussion

Drug interactions may have pharmacokinetic or/and pharmacodynamic origins, and for that reason pharmacodynamic interactions alone are difficult to distinguish *in vivo*. However an interesting situation occurs when drug concentrations can be measured within the biophase at the onset of activity, because it makes possible the differentiation between the pharmacokinetic and pharmacodynamic contributions to the
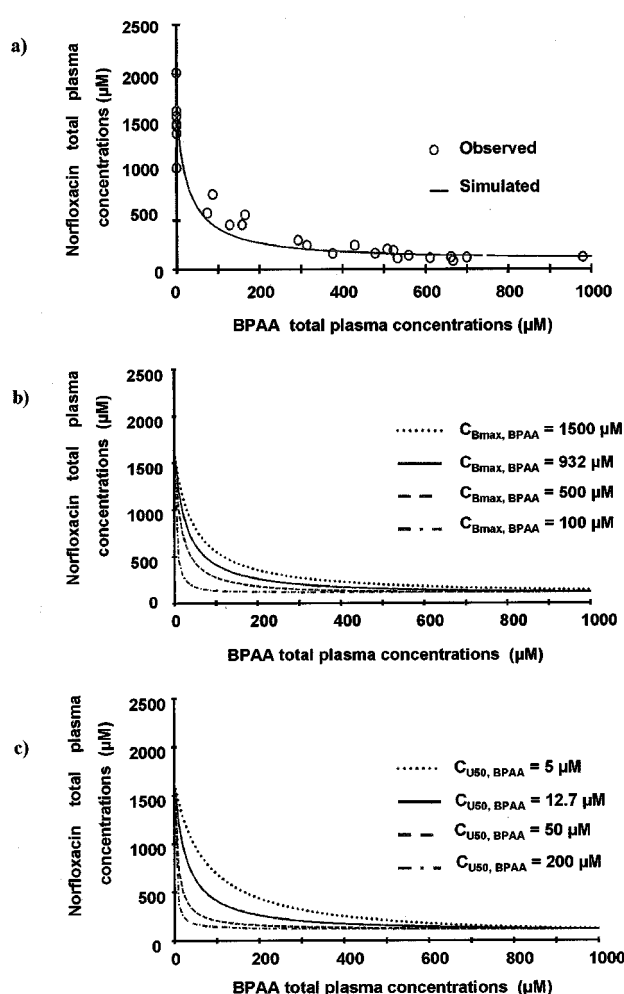

Figure 6 Norfloxacin versus BPAA concentrations in total plasma. (a) was obtained by superimposition of simulated curve obtained from Equation 7 and experimental data points. Norfloxacin versus BPAA total plasma concentrations were simulated for various values of the parameters characteristic of the non-linear protein binding of BPAA: $C_{B_{max}, BPAA}$ from 100 μM (bottom, left) to 1500 μM (top, right) (b) and $C_{U50, BPAA}$ from 5 μM (top, right) to 200 μM (bottom, left) (c).

observed effect. The cyclosporine-theophylline convulsant interaction has been investigated with such an approach (Hoffman *et al.*, 1994). However cyclosporine was administered at a fixed dose, precluding full characterization of the interaction, as could be done with more complete experimental protocols (Zhi & Levy, 1990; Levasseur *et al.*, 1998; Delon *et al.*, 1999b).

The convulsant interaction between BPAA and norfloxacin was therefore investigated with an experimental approach similar to that previously used for the characterization of the pefloxacin-theophylline (Levasseur *et al.*, 1998) and pefloxacin-norfloxacin (Delon *et al.*, 1999b) convulsant interactions. The basic concept consists in measuring the concentrations of the two drugs in CSF, which was previously shown to be part of the biophase (Delon *et al.*, 1997) at the onset of activity. However in these previous studies, the two compounds had a convulsant effect on their own, whereas in the present situation only norfloxacin may induce convulsions, BPAA having only a pro-convulsant effect. Therefore the isobolographic approach previously used, was not appropriate to investigate the BPAA-norfloxacin interaction and a new model had therefore to be developed.

The pro-convulsant effect of BPAA was well characterized in the biophase (CSF) by an inhibitory E_{max} effect model with a

baseline effect parameter. At first glance this model (Equation 1) could also apply to free plasma concentrations, except for the $C_{Ubase, Nor}$ which could not be estimated accurately. Furthermore, this modelling would suggest that the pro-convulsant effect of BPAA increases the convulsant activity of norfloxacin by up to 18 times (ratio between $C_{U0, Nor}$ and $C_{Ubase, Nor}$) when CSF data indicated an increase by 6 fold at the most (ratio between $C_{CSF0, Nor}$ and $C_{CSFbase, Nor}$). This is because the same structural model (Equation 1) can apply to both CSF and free plasma concentration data sets, only if the CSF diffusion of the two compounds are linear, which was not the case (Figure 3). Therefore a new relationship (Equation 4) had to be found between unbound plasma concentrations, taking into account the linear CSF diffusion of BPAA and the non-linear CSF diffusion of norfloxacin. The originality of this approach summarized on Figure 1, was to consider that although CSF concentrations are determined by plasma concentrations (Delon *et al.*, 1997), the effect (convulsions) appears when BPAA and norfloxacin concentrations reach certain values in CSF (biophase) independently of plasma levels. Therefore there is a direct relationship between the CSF concentrations of the two drugs at the onset of activity, which in fact constitutes the driving force of the system. Plasma concentrations are only indirectly related.

The apparent non-linear CSF diffusion of norfloxacin (Equation 2 and Figure 3a), may have several origins, including the existence of some active transport systems (Jaehde *et al.*, 1992), a pharmacokinetic (CSF diffusion) interaction with BPAA (Ichikawa *et al.*, 1992), and a relatively slow distribution of norfloxacin into CSF (Delon *et al.*, 1997) together with a decrease in infusion time when BPAA doses increased (Table 1). This effect of the non-linear CSF diffusion of norfloxacin on the relationship between the unbound plasma concentrations of norfloxacin and BPAA was further investigated using computer simulations with various values of $C_{CSF_{max}, Nor}$ and $C_{U50, Nor}$ (Figure 5b,c). Simulations showed that changes in $C_{CSF_{max}, Nor}$ or $C_{U50, Nor}$ do not really affect the general shape of the curve, which is only shifted to the top (or right side) when $C_{CSF_{max}, Nor}$ decreases (Figure 5b) or when $C_{U50, Nor}$ increases (Figure 5c). This observation is consistent with the fact that a reasonably good data fitting of unbound plasma concentrations could be obtained with Equation 1. However as can also be observed and deduced from Equation 4, the ratio $C_{U0, Nor}/C_{Ubase, Nor}$ does not change with $C_{U50, Nor}$, but varies with $C_{CSF_{max}, Nor}$. This explains the inconsistency between the estimates of the $C_{CSF0, Nor}/C_{CSFbase, Nor}$ and $C_{U0, Nor}/C_{Ubase}$ ratios, obtained when Equation 1 was applied to both sets of data.

As for unbound concentrations, an indirect relationship was derived (Equation 7) between total plasma concentrations of the two drugs (Figure 6a), taking into account the various direct relationships identified and illustrated on Figure 1, in particular the nonlinear plasma protein binding of BPAA (equation 5a). Parameters characteristic of this non-linear protein binding ($C_{B_{max}, BPAA}$ and $Cu_{50, BPAA}$) were then let to vary in order to estimate their effect on the indirect relationship between the total plasma concentrations of the two drugs. As illustrated on Figure 6b,c, $C_{P0, Nor}$ and $C_{Pbase, Nor}$ are not affected by changes in $C_{B_{max}, BPAA}$ or $Cu_{50, BPAA}$ However the general shape of the $C_{P, Nor}$ versus $C_{P, BPAA}$ curves is modified. The curvature becomes less pronounced as $C_{B max, BPAA}$ increases or $Cu_{50, BPAA}$ decreases. Therefore the saturable protein binding of the pro-convulsant compound is the main reason why the initial inhibitory model (Equation 1) was not appropriate to fit the total plasma concentrations data sets.

In conclusion the pro-convulsant effect of BPAA on norfloxacin as a representative FQ, has been investigated for the first time *in vivo*, with an approach distinguishing between the pharmacokinetics and the pharmacodynamics of this interaction. It was shown that the pharmacodynamic interaction could be well and best characterized from the CSF (biophase) concentration measurements at the onset of activity, providing that the two drugs had been administered in a sufficiently large range of doses in order to estimate adequately the three parameters characteristic of the interaction model. Plasma concentrations alone can hardly provide such information, because of pharmacokinetic complexities such as the non-linear plasma protein binding of BPAA, or the apparent non-linear CSF diffusion of the FQ. Interestingly unbound plasma concentrations, which are frequently considered as the 'active form' of the drugs, do not properly reflect the interaction in this particular situation as well. Ignoring these pharmacokinetic complexities would lead to false conclusions when plasma concentrations are considered alone, and to major inconsistencies when they are compared to the analysis of CSF concentrations. The integrated pharmacokinetic-pharmacodynamic modelling procedure, successfully developed to reconcile these apparently conflicting data, is now being used in our laboratory to compare the pro-convulsant effect of BPAA on various FQs.

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