Pharmacokinetic-Pharmacodynamic Contributions to the Convulsant Activity of Pefloxacin and Norfloxacin in Rats

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ABSTRACT
The purpose of this investigation was to compare the convulsant activity of two quinolones differing, respectively, by the presence (pefloxacin) or absence (norfloxacin) of a methyl group on the piperazine moiety at the position 7 of their parent nuclei and consequently by their lipophilicity. An in vivo model was used, which can distinguish between ease in reaching pharmacological receptors at the central nervous system level, and ability to interact with these receptors. Male Sprague-Dawley rats (~230g-300g) received an i.v. infusion of pefloxacin or norfloxacin at one of four different rates: 480, 960, 1440 and 1920 μmol/hr, until the onset of maximal seizures. This occurred after an average of 12.7 to 69.4 min. We found enough evidence to suggest that in these conditions the contribution of pefloxacin metabolites, including norfloxacin, to its convulsant activity was negligible. Doses of pefloxacin and concentrations in cerebrospinal fluid and plasma (total and unbound) at the pharmacodynamic endpoint were all independent of infusion rate, whereas only cerebrospinal fluid concentrations of norfloxacin were independent of infusion rate. The overall cerebrospinal fluid concentration of norfloxacin (47.3 ± 9.9 μmol/liter) was about 8-fold lower than that of pefloxacin (380 ± 27 μmol/liter), indicating that on average the “intrinsic convulsant activity” of norfloxacin is 8-fold greater than that of pefloxacin. However, total doses of pefloxacin and norfloxacin at the onset of maximal seizures were in the same order of magnitude (1500–2000 μmol/kg), suggesting that the higher ability of the more lipophilic pefloxacin to reach central nervous system compensates for its lower intrinsic convulsant activity.

Quinolone carboxylic acid derivatives (quinolones) have been primarily used in the treatment of urinary infections. More recently, the widespread use of new fluorinated quinolones in the treatment of various bacterial infections has proven to be highly effective, on account of their broad and strong activity against gram-negative bacteria, including those resistant to aminoglycoside and β-lactam antibiotics (Janknegt, 1986). Because quinolones can distribute into the CNS, they have been proposed as an alternative in the treatment of CNS infections (Scheld, 1989). However, several CNS side effects of quinolones have been reported including headache, confusion, hallucination, anxiety, nervousness, nightmares and seizures (Christ, 1990). The incidence of CNS side effects is nonetheless quite low, but seizures have been observed most frequently in patients receiving quinolones in combination with nonsteroidal antiinflammatory drugs such as fenbufen (Simpson and Brodie, 1985; Arcieri et al., 1987; Anastasio et al., 1988).

The mechanism by which quinolones exhibit amplified epileptogenic activity in combination with BPAA is not clear, but it has been proposed that CNS excitation could result from inhibition of GABA_A binding to its receptors (Segev et al., 1988; Akahane et al., 1989). Although the convulsant activity of quinolones can be reproduced in animals, especially when administered concomitantly with BPAA, most of the published data on the relationship between the chemical structure and the epileptogenic activity of quinolones, have been obtained with special reference to the in vitro interaction with GABA receptor sites (Tsuji et al., 1988; Akahane et al., 1994). The absence or presence of a methyl group on the piperazine moiety at the 7 position of the parent nuclei, should be one of the major determinants of quinolone neurotoxicity (Domagala, 1994). Although in vitro studies provided valuable information, their extrapolation onto the in vivo situation, in particular when quinolones are administered without BPAA, remains questionable (Akahane et al., 1994).

The individual fluoroquinolones considerably differ in their physicochemical characteristics, pharmacokinetic properties and therefore CSF penetration (Sörgel et al., 1987), which is likely to be another important factor of neurotoxicity insofar as quinolones concentrations in CSF are likely to be related to the incidence of CNS side effects (Bonati et al., 1982).

The objective of this study was to assess the applicability of the experimental approach developed by Levy and collabora-

ABBREVIATIONS: BPAA, biphenylacetic acid; CSF, cerebrospinal fluid; CNS, central nervous system; GABA, γ-aminobutyric acid; UF, ultrafiltrate.
tors (Danhof and Levy, 1984) and subsequently used with several drugs, including compounds inducing convulsions, such as pentylentetrazol (Ramzan and Levy, 1985) and theophylline (Ramzan and Levy, 1986), to investigate the relative contributions of the pharmacokinetic (ability to reach receptors) and pharmacodynamic (affinity for these receptors) characteristics of quinolones, to their in vivo convulsant activity. Pefloxacin was selected as a probe drug because of its expected high CNS penetration due to its relative high lipophilicity, combined with low convulsant activity due to the presence of a methyl group on the piperazine moiety at the 7 position of its parent nuclei. By contrast, norfloxacin was chosen because of its presumably high convulsant activity due to the absence of methyl group, combined with low CNS penetration due to relatively low lipophilicity.

Materials and Methods

Animals

Animals were housed in the Animal Breeding Facilities of the Laboratory (authorization no. 0028). In the first part of this study, male Sprague-Dawley rats (Depres Breeding Laboratories, St. Doulchard, France) were used to study the effect of infusion rate on the pharmacodynamics of pefloxacin and norfloxacin. Their body weights ranged from 230 to 300 g with an average of 267 ± 17 g (mean ± S.D.). The animals were housed in wire cages in a 12hr light-dark cycle for 1 wk to adjust to the new environment and to overcome possible stress incurred during transit. They had free access to food (Extralabo M20, Pietremont Laboratories, Provins, France) and water. In a second part, “inter-occasions experiments” were conducted to investigate between groups/between days variability of the pharmacodynamic response of pefloxacin using animals from three different breeding centers: Charles Rivers (Saint-Eubin-Lès-Elbeuf, France), Iffa Credo (Domaine de Oncins, L’Arbresle, France), and Depres.

Surgery

Animals had a cannula implanted in the left jugular vein 1 day before the experiment. They were anesthetized with 60 mg/kg sodium pentotal (Sanofi Laboratories, Gentilly, France). A lateral incision was made on the ventral surface of the neck and the left jugular vein exposed using blunt dissection. The vessel was teed at the anterior end and a hole punctured posterior to it. A polyurethane catheter (0.58-mm inside; 0.98-mm outside diameter, Plastimed Laboratories, Saint-Leu-La-Forêt, France) was then inserted into the hole and pushed 2.5 to 3 cm toward the heart. This catheter was secured to the vessel by a suture tied around it. The line was cleared with a saline solution of heparin (100 IU/ml) and plugged to maintain potency. The catheter was brought through a tunnel between the skin and underlying muscles and externalized between the two shoulder blades. Animals were housed individually in plastic cages. Food was withdrawn 12 hr before the experiment, but the animals had free access to water until drug infusion.

Solutions for Administration

A commercially available solution of pefloxacin methane sulfonate (Roger Bellon Laboratories, Neuilly Sur Seine, France) was used for administrations. Its concentration was equal to 80 mg/ml, corresponding to 240 mmol/liter of pefloxacin. A norfloxacin salt was prepared by dissolving norfloxacin (Sigma, Saint-Quentin Fallavier, France) in anhydrous acetone followed by precipitation with a saturated solution of chlorhydric alcohol. After filtration the precipitate was dried in a vacuum desiccator. A solution of norfloxacin hydrochloride in 5% glucose, titrating 76.6 mg/ml (corresponding to 240 mmol/liter) with a pH of 5.5, was prepared for administration.

Drug Administration

The day after surgery, the jugular vein cannula was connected to a motor-driven syringe pump (SER202B, Vial Inc., Saint-Etienne de Saint-Geoirs, France) containing the drug solution (pefloxacin or norfloxacin). Animals were kept under heating lamp to maintain body temperature. The infusion was stopped when the animal 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 2:00 and 6:00 P.M. The volume of solution administered varied from 1.5 to 2.5 ml.

Study Design

In the first series of experiments, the effect of infusion rate on the concentration of quinolones in CSF, plasma (total and free) and brain, at the onset of maximum seizures was determined in rats who received an i.v. infusion of pefloxacin or norfloxacin at one of the following rates: 480, 960, 1440 and 1920 µmol/hr (corresponding to 2, 4, 6 and 8 ml/hr) according to a randomized trial. In a second part, “inter-occasions experiments” were conducted to investigate between groups/between days variability of the pharmacodynamic response of pefloxacin administered at a rate of 960 µmol/hr, as follows. Occasion A: animals from Depres Breeding Center were used in January 1994, occasion B: animals from Charles Rivers in July 1994 and occasion C: animals from Iffa Credo in October 1994.

Sample Collection

Immediately after exhibiting maximal seizures, rats were anesthetized with an intramuscular injection of 12.5 mg of ketamin (KETALAR, 50 mg/ml, Parke Davis Laboratories, Courbevoie, France) and 5 mg of xylazin hydrochloride (ROMPIN, Bayer Laboratories, Sene, France), unless they had died following maximal seizures. Samples were collected in this order: CSF, blood, brain, within 3, 5 and 6 min, respectively, after the end of infusion.

CSF samples. Animals were placed on a stereotaxic apparatus. Crystal-clear CSF specimens were obtained by cisternal puncture. Upon flexing the head acutely, the external occipital protuberance was thrown into prominence. Directly caudal to this, a depression was felt between the protuberance and the spine of the atlas. The needle was thrust into the center of this depression. As it entered the cisterna magna there was a decrease in resistance and the CSF could rise by aspiration in the tubing (Microflex Infusion Set: 0.5 mm/G.25, Vygon Laboratories, Ecouen, France).

Plasma samples. Immediately after CSF collection, blood was obtained from the abdominal aorta and collected in heparinized tubes (VACUTAINER). It was immediately centrifuged at 1000 x g for 10 min (GR412 model, Jouan) and 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, Epernon, France) for determination of free concentrations.

Brain samples. After decapitation by guillotine, brains were quickly removed from the skull and rinsed with a solution of NaCl 0.9% and stored at −20°C until analysis.

Drug Analysis

A previously described high performance liquid chromatography assay (Jaehde et al., 1992) was used with minor modifications. The parent drugs (pefloxacin or norfloxacin) were assayed in CSF and UP by direct injection after dilution (1:10) in a mixture of 0.1 M citrate buffer (pH = 3) including pipemidic acid as the internal standard. Plasma samples were diluted (1:25) by addition of a 1.7% (v/v) perchloric acid/pipemidic acid mixture. Subsequently the mixture was centrifuged and 20 µl of the supernate were injected onto the column. Brains were accurately weighed and homogenized in 5 ml of 0.1 mM citrate buffer (pH = 3) with pipemidic acid, for 3 min (Ultraturax T25, Janke and Kunkel) at 18,000 rpm. The homoge-
rates were centrifuged at 2000 × g for 15 min and 20 μl of the supernatant were injected onto the column. Pefloxacin metabolites (norfloxacin and N-oxide pefloxacin) were assayed in CSF and UF by direct injection, and in plasma after deproteinization with an equal volume of perchloric acid 1.7%. Separation was performed with a Spherisorb ODS (5 μm, 300 × 0.4 mm inner diameter) column. The mobile phase consisted of 0.1 M aqueous citric acid solution containing 13% (v/v) acetonitrile and 10 mM tetra butyl ammonium perchlorate and the flow rate was 0.8 ml/min. Retention times of piper- midic acid, norfloxacin, pefloxacin and N-oxide pefloxacin were respectively equal to 5.8, 11.4, 14.3 and 18.9 min. The chromatographic system consisted of a model 510 pump and a Waters (Saint-Quentin en Yvelines, France) 712 autosampler connected to a fluoriometric detector (excitation wavelength = 280 nm, emission wavelength = 450 nm). A Kratos 980 fluorimeter with a deuterium lamp was used for the determination of unchanged pefloxacin and norfloxacin, and a Waters 470 fluorimeter with xenon lamp for the analysis of pefloxacin metabolites. Chromatographic data were recorded and processed using a Waters 746 integrator. Calibration was performed by adding known amounts of pefloxacin, norfloxacin or N-oxide pefloxacin to blank plasma, NaCl 0.9% solution (for UF and CSF) or brain at appropriate concentrations. The limit of quantification was 0.5 μg/ml in plasma, UF and CSF and 1.0 μg/g of brain for parent compounds. It was 0.1 μg/ml in plasma and 0.05 μg/ml in UF and CSF for pefloxacin metabolites. The relative error and the intra-day coefficient of variation at the limit of quantification were respectively less than ± 20 and 10% (or 20% in brain). Intra-day coefficients of variation calculated for each compound at two concentrations close to the usually measured values, were equal to or less than 3.3, 4.2, 4.5 and 11.2% in plasma, UF, CSF and brain, respectively. Corresponding inter-day coefficients were equal to or less than 7.1, 9.8, 9.5 and 15.0%.

Statistical Analysis

Results are expressed as mean ± S.D. Doses, calculated as the product of the infusion time by the rate of infusion, and concentrations were compared statistically by analysis of variance followed by a Student’s t test where appropriate. Bartlett’s test was used to assess homogeneity of variances. Some of the results of these tests were confirmed by nonparametric analysis (Mann and Whiney test).

Results

In the first part of this study, pefloxacin and norfloxacin were infused i.v. at four different rates ranging from 480 to 1920 μmol/hr. Infusion duration ranged from 11.0 to 74.0 min (pefloxacin) and from 10.2 to 81.6 min (norfloxacin), depending on the rate. The effects of infusion rate on total dose, total and unbound plasma concentrations, CSF and brain concentrations at onset of maximal seizures are summarized in tables 1 and 2.

Total dose of pefloxacin at the onset of maximal seizures tended to decrease when infusion rate increased, but not significantly. Corresponding concentrations in brain did not vary significantly when the infusion rate increased from 480 to 1440 μmol/hr (overall average concentration equal to 357 ± 44 μmol/kg), but rose significantly (452 ± 26 μmol/kg) at the highest infusion rate (1920 μmol/hr). Total and unbound plasma concentrations tended to increase with infusion rate, but once again not significantly. Pefloxacin CSF concentrations at onset of maximal seizures were markedly independent of the infusion rate. The overall average concentration in CSF (n = 28), was equal to 380 ± 27 μmol/liter (or 127 ± 9 μg/ml). The free fraction of pefloxacin in plasma was independent of the infusion rate and equal to 60 ± 6.5% on average. Metabolite to parent drug concentration ratios determined in plasma samples (n = 22) are listed in table 3. Several CSF samples obtained for investigation of inter-occasion variability (second part of this study) could also be used for metabolite determinations. The mean corresponding norfloxacin to pefloxacin concentrations ratio was equal to 0.32 ± 0.10% (n = 15), and the mean N-oxide derivative to pefloxacin concentration ratio to 0.27 ± 0.19% (n = 12), meaning that CSF pefloxacin concentrations were at least 200-fold higher than corresponding metabolite levels.

Total dose of norfloxacin at the onset of maximal seizures decreased significantly (P < .01) from 1996 ± 304 to 1476 ± 294 μmol/kg when infusion rate increased from 480 to 1920 μmol/hr (table 2). Corresponding concentrations in plasma (both total and unbound) varied significantly with the infusion rate. In contrast, norfloxacin concentrations in CSF at onset of maximal seizures were independent of the infusion rate and equal on average (n = 25) to 47.3 ± 9.9 μmol/liter (or 15.1 ± 3.2 μg/ml). Finally, the free fraction of norfloxacin in plasma was independent of the infusion rate and equal to 82 ± 11% on average.

The doses of pefloxacin at the onset of maximal seizures obtained on occasions A, B and C, of the “inter-occasions experiments,” were, respectively, equal to 1573 ± 143, 1876 ± 201 and 1920 ± 263 μmol/kg.

### TABLE 1

Effect of pefloxacin infusion rate on total dose and concentrations of pefloxacin in plasma, brain and CSF at onset of maximum seizure in rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>480</th>
<th>960</th>
<th>1440</th>
<th>1920</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infusion Rate (μmol/hr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total dose (μmol/kg)</td>
<td>1876 ± 201</td>
<td>1813 ± 151</td>
<td>1851 ± 178</td>
<td>1678 ± 160</td>
</tr>
<tr>
<td>Total plasma concentration (μmol/liter)</td>
<td>771 ± 62</td>
<td>778 ± 69</td>
<td>817 ± 109</td>
<td>895 ± 113</td>
</tr>
<tr>
<td>Free plasma concentration (μmol/liter)</td>
<td>456 ± 53</td>
<td>486 ± 57</td>
<td>492 ± 30</td>
<td>518 ± 38</td>
</tr>
<tr>
<td>CSF concentration (μmol/liter)</td>
<td>377 ± 17</td>
<td>377 ± 35</td>
<td>374 ± 31</td>
<td>388 ± 26</td>
</tr>
<tr>
<td>Free (unbound) fraction in plasma</td>
<td>0.60 ± 0.07</td>
<td>0.60 ± 0.05</td>
<td>0.61 ± 0.08</td>
<td>0.57 ± 0.06</td>
</tr>
<tr>
<td>CSF/free plasma concentration ratio</td>
<td>0.83 ± 0.07</td>
<td>0.80 ± 0.13</td>
<td>0.76 ± 0.10</td>
<td>0.74 ± 0.06</td>
</tr>
<tr>
<td>Brain concentration (μmol/kg)</td>
<td>357 ± 39</td>
<td>351 ± 52</td>
<td>372 ± 42</td>
<td>452 ± 26</td>
</tr>
</tbody>
</table>

Results are reported as mean ± SD.

* Infusion rate had a significant effect (P < .001 by one-way analysis of variance) on brain concentrations, but not on total dose, concentrations in plasma (total and unbound) and CSF, unbound fraction in plasma and CSF/free plasma concentration ratios.

* n = 8 due to failure to obtain blood from one rat.

* Significant different from other infusion rates (P < .001 by Student’s t test).

### TABLE 2

Effect of norfloxacin infusion rate on total dose and concentrations of norfloxacin in plasma, brain and CSF at onset of maximum seizure in rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>480</th>
<th>960</th>
<th>1440</th>
<th>1920</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infusion Rate (μmol/hr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total dose (μmol/kg)</td>
<td>1876 ± 201</td>
<td>1813 ± 151</td>
<td>1851 ± 178</td>
<td>1678 ± 160</td>
</tr>
<tr>
<td>Total plasma concentration (μmol/liter)</td>
<td>771 ± 62</td>
<td>778 ± 69</td>
<td>817 ± 109</td>
<td>895 ± 113</td>
</tr>
<tr>
<td>Free plasma concentration (μmol/liter)</td>
<td>456 ± 53</td>
<td>486 ± 57</td>
<td>492 ± 30</td>
<td>518 ± 38</td>
</tr>
<tr>
<td>CSF concentration (μmol/liter)</td>
<td>377 ± 17</td>
<td>377 ± 35</td>
<td>374 ± 31</td>
<td>388 ± 26</td>
</tr>
<tr>
<td>Free (unbound) fraction in plasma</td>
<td>0.60 ± 0.07</td>
<td>0.60 ± 0.05</td>
<td>0.61 ± 0.08</td>
<td>0.57 ± 0.06</td>
</tr>
<tr>
<td>CSF/free plasma concentration ratio</td>
<td>0.83 ± 0.07</td>
<td>0.80 ± 0.13</td>
<td>0.76 ± 0.10</td>
<td>0.74 ± 0.06</td>
</tr>
<tr>
<td>Brain concentration (μmol/kg)</td>
<td>357 ± 39</td>
<td>351 ± 52</td>
<td>372 ± 42</td>
<td>452 ± 26</td>
</tr>
</tbody>
</table>

Results are reported as mean ± SD.

* Infusion rate had a significant effect (P < .001 by one-way analysis of variance) on brain concentrations, but not on total dose, concentrations in plasma (total and unbound) and CSF, unbound fraction in plasma and CSF/free plasma concentration ratios.

* n = 8 due to failure to obtain blood from one rat.

* Significant different from other infusion rates (P < .001 by a Student’s t test).
TABLE 2
Effect of norfloxacin infusion rate on total dose and concentrations of norfloxacin in plasma, brain and CSF at onset of maximum seizure in rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>480</th>
<th>960</th>
<th>1440</th>
<th>1920</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>7</td>
<td>8</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>280 ± 14</td>
<td>280 ± 8</td>
<td>275 ± 14</td>
<td>282 ± 11</td>
</tr>
<tr>
<td>Infusion time (min)</td>
<td>69.4 ± 8.9</td>
<td>34.5 ± 4.7</td>
<td>19.7 ± 2.9</td>
<td>12.9 ± 2.3</td>
</tr>
<tr>
<td>Total dose (μmol/kg)</td>
<td>1996 ± 304</td>
<td>1966 ± 253</td>
<td>1750 ± 275</td>
<td>1476 ± 294ab</td>
</tr>
<tr>
<td>Total plasma concentration (μmol/liter)</td>
<td>1103 ± 196cde</td>
<td>1400 ± 258def</td>
<td>1836 ± 335g</td>
<td>1646 ± 610</td>
</tr>
<tr>
<td>Free plasma concentration (μmol/liter)</td>
<td>682 ± 222ghi</td>
<td>1146 ± 169jkl</td>
<td>1490 ± 308ab</td>
<td>1277 ± 437</td>
</tr>
<tr>
<td>CSF concentration (μmol/liter)</td>
<td>49.2 ± 6.6qrs</td>
<td>48.2 ± 11.9uvt</td>
<td>42.6 ± 7.5wxy</td>
<td>50.4 ± 14.7z</td>
</tr>
<tr>
<td>Free (unbound) fraction in plasma</td>
<td>0.82 ± 0.14</td>
<td>0.84 ± 0.11</td>
<td>0.81 ± 0.07</td>
<td>0.81 ± 0.14</td>
</tr>
<tr>
<td>CSF/free plasma concentration ratio</td>
<td>0.063 ± 0.025</td>
<td>0.042 ± 0.011</td>
<td>0.031 ± 0.008</td>
<td>0.038 ± 0.008</td>
</tr>
<tr>
<td>Brain concentration (μmol/kg)</td>
<td>87 ± 21</td>
<td>113 ± 27</td>
<td>129 ± 13</td>
<td>113 ± 31</td>
</tr>
</tbody>
</table>

Results are reported as mean ± SD.

a Infusion rate had a significant effect (p < .05 by one-way analysis of variance) on infusion time, total dose and concentrations in plasma (total and unbound), but not on CSF and brain concentrations, and unbound fraction in plasma.

b Significantly different from corresponding doses at 480 and 960 μmol/hr infusion rates (P < .001 by a Student’s t test).

c Significantly different from corresponding doses at 480 and 960 μmol/hr infusion rates (P < .001 by a Student’s t test).

d Significantly different from other infusion rates (P < .05 by a Student’s t test), except for the 1920 μmol/hr infusion rates (Mann-Whitney U test).

e n = 6.

f n = 7.
g Significantly different from corresponding doses at 480 and 960 μmol/hr infusion rates (P < .05 by a Student’s t test).

TABLE 3
Metabolites to pefloxacin concentrations ratios (%) in plasma at onset of maximum seizure in rats

<table>
<thead>
<tr>
<th>Rates of Infusion (μmol/hr)</th>
<th>Norfloxacin</th>
<th>N-Oxide Pefloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma</td>
<td>Plasma</td>
</tr>
<tr>
<td>480</td>
<td>0.72 ± 0.28</td>
<td>1.79 ± 0.38</td>
</tr>
<tr>
<td>960</td>
<td>0.50 ± 0.15</td>
<td>1.20 ± 0.35</td>
</tr>
<tr>
<td>1440</td>
<td>0.36 ± 0.22</td>
<td>0.71 ± 0.20</td>
</tr>
<tr>
<td>1920</td>
<td>0.42 ± 0.27</td>
<td>0.91 ± 0.48</td>
</tr>
</tbody>
</table>

Results are expressed as percentage, and reported as mean ± S.D.

1617 ± 210 and 1640 ± 170 μmol/kg (not significantly different). Corresponding CSF concentrations were equal to 380 ± 29, 366 ± 12 and 333 ± 21 μmol/liter (P < .01).

Discussion
The major findings of this investigation concern 1) evaluation of the ability of a previously published model to investigate the convulsant activity of quinolones in rats in vivo, 2) determination of optimum sampling site(s) for these two compounds with different lipophilicity and 3) evaluation of the relative potencies of these two compounds differing only by the presence (pefloxacin) or the absence (norfloxacin) of a methyl group on the piperazine moiety at the 7 position of their parent nuclei.

Ability of the model to investigate the convulsant activity of quinolones. Using solutions of pefloxacin mesylate and norfloxacin hydrochloride at relatively high concentrations (240 mmol/liter), it was possible to provoke convulsions in rats after administration of a reasonably low injected volume. Because pharmacologically active metabolites complicate data interpretation, their presence in the different biological fluids of interest requires cautious investigation (Ramzan and Levy, 1986; Klockowski and Levy, 1988). Pefloxacin is metabolized to norfloxacin and to an N-oxide derivative in different species including man and rat (Montay et al., 1984). Because the longest infusion time used in our investigations (74.0 min) was quite short (about one-fourth) compared with pefloxacin elimination half-life (Jaehde et al., 1992), formation and accumulation of metabolites could not be extensive. However, these metabolites had previously been found in the plasma and CSF of rats after i.v. administration of 4 mg of pefloxacin, with norfloxacin to pefloxacin area under the curve ratios equal to 4.5 ± 0.3% in plasma and 12.5 ± 8.2% in CSF, and N-oxide metabolite to pefloxacin ratios equal to 8.2 ± 1.5% and 5.7 ± 2.6% (Jaehde et al., 1992). We obtained much lower metabolite/parent drug ratios that may be due to the difference between doses (30-fold ratio), but more importantly we report ratios between levels measured at an early time, when distribution equilibrium may not have been reached, and they cannot be directly compared with area under the curve ratios.

The observation that CSF pefloxacin concentrations were at least 200-fold higher than corresponding metabolite levels, suggests that most of the convulsant activity was due to unchanged pefloxacin. The infusion rate independence of pefloxacin concentrations in CSF at the onset of maximal seizures, is a further indication that metabolites do not accumulate during infusion and contribute little or not at all to the seizures produced by pefloxacin infusions (Ramzan and Levy, 1985). It is also an indirect proof of the absence, during infusion, of acute tolerance development, which would have precluded the applicability of this experimental approach (Ramzan and Levy, 1985).

Determination of optimum sampling site. The administered doses of pefloxacin, as well as its concentrations in plasma and CSF of rats at the onset of maximal seizures, did not vary significantly with infusion rate, indicating that pefloxacin equilibrates very rapidly between its action sites and plasma. As opposed to pefloxacin, the dose and plasma concentrations of norfloxacin at the onset of maximal seizures were significantly affected by the infusion rate. However, norfloxacin concentrations in CSF at the onset of maximal seizures were independent of the infusion rate. Such a concentration "overshoot" in plasma at the more rapid infusion rate had previously been observed with theophylline (Ramzan and Levy, 1986), and indicates relatively slow distribution of the drug into CSF. The difference between pefloxacin and norfloxacin rates of penetration in CSF is also
illustrated by the CSF to unbound plasma concentration ratios, equal to 74% at least for pefloxacin (table 1), and to 6.3% at most for norfloxacin (table 2).

These results are consistent with data obtained in cell culture models, and should be explained by the differences in lipophilicity between these two drugs (Jaechde et al., 1993). As a consequence, under these experimental conditions and from a pharmacokinetic perspective, CSF and plasma reflect concentrations in the biophase almost equally well in the case of pefloxacin, but only norfloxacin CSF levels reflect concentrations in the biophase.

Relative potencies of pefloxacin and norfloxacin. The CSF concentration of norfloxacin at the onset of maximal seizures (47.3 ± 9.9 μmol/liter) was 8-fold less, on average, than the corresponding value for pefloxacin (380 ± 27 μmol/liter). Assuming that in these experimental conditions, pefloxacin metabolites do not contribute to the convulsant activity of the drug, we may conclude that what we define as the intrinsic convulsant activity of norfloxacin, is 8-fold higher on average than that of pefloxacin. This is consistent with the currently accepted idea that quinolones with a piperazine substituted with a methyl group at the 7 position, are less convulsant than corresponding unsubstituted derivatives (Domagala, 1994). However, such notions have been derived from in vitro binding experiments, in which the affinity of various quinolones for GABA_A receptors, was evaluated through their inhibitory activity on [3H]muscimol binding in the presence of BPAA (Akahane et al., 1989). These in vitro experiments have also suggested that exceptions to this rule may exist, such as with lomefloxacin (Aka-hane et al., 1989). More importantly, because of the presence of BPAA, these data may not be predictive of the convulsant activity of quinolones alone (Akahane et al., 1994). Finally, another limitation in these in vitro binding experiments is that other neurotransmitter systems such as the glutamate receptors, may also be involved in the convulsant activity of quinolones (Dodd et al., 1989).

The weaker intrinsic convulsant activity of pefloxacin that we observed, may easily be attributed to the presence of a methyl group at the 7 position of the piperazino moiety, because this is its only structural difference with norfloxacin. Interestingly, the total doses of pefloxacin and norfloxacin administered to produce maximal seizures were in the same order of magnitude (between 1500 and 2000 μmol/kg), indicating that the differences in pharmacokinetic and pharmacodynamic contributions to the convulsant activity, compensate almost perfectly for these two quinolones.

The inter-occasion experiment demonstrated relatively low yet significant differences between CSF concentrations at the onset of maximal seizures, using rats from different breeding centers at different periods. These data are consistent with results previously observed with diazepam (Klockowski and Levy, 1988). They suggest that historical data should not be used for rigorous comparisons, and that experiments should be limited in time.

In conclusion, determination of quinolone concentrations in CSF at the onset of maximal seizures after i.v. infusion of pefloxacin or norfloxacin has been validated in this first study, as a very useful experimental strategy for the assessment of their convulsant activity in rats in vivo. We have demonstrated 1) that norfloxacin is on average intrinsically 8-fold more convulsant than pefloxacin, due to the loss of a methyl group on the piperazino moiety at the 7 position of the parent nuclei, 2) that this greater intrinsic activity is compensated for by a difference in ability to reach CSF, probably due to a difference in lipophilicity between the two compounds. Therefore the intrinsic convulsant activity of quinolones, and their ability to reach CNS, are two major determinants of their neurotoxicity in vivo. These data appear very promising and in a subsequent report we will describe, using the same methodology, a more complete structure-convulsant activity study of quinolones.

References


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