A New Approach for Early Assessment of the Epileptogenic Potential of Quinolones

ANNIE DELON, CLAUDINE PARIAT, PHILIPPE COURTOIS, SERGE BOUQUET, and WILLIAM COUET*

UPRES-EA 1223, Faculté de Médecine et Pharmacie, Poitiers, France

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The epileptogenic potential of pefloxacin and norfloxacin, two quinolone antibiotics, was investigated in vivo in three different animal species by measuring drug concentrations in cerebrospinal fluid (CSF), which is part of the biophase, at the onset of convulsions. Interestingly, the pefloxacin-to-norfloxacin concentration ratios in CSF were virtually constant across the species (7.0, 6.6, and 6.0 in mice, rats, and rabbits, respectively), suggesting that this approach could be used to predict the relative epileptogenic potential of quinolones in humans.

Numerous quinolones are on the market, and several others are in development. Although these antibiotics are generally well tolerated, central nervous system (CNS) toxicity may occur, including headache, confusion, hallucination, anxiety, nervousness, nightmares, and even seizures (5). It is therefore important to characterize the epileptogenic potential of new quinolones as soon as possible during their preclinical development. This is usually assessed in vitro by determination of the affinity for γ-aminobutyric acid A (GABA_A) receptors (1, 16). However, the extrapolation of these results to the clinical setting seems very difficult for several different reasons. First, such an approach assumes that the epileptogenic activity is directly and strictly related to inhibition of GABA_A receptors, which may not be the case (4). Furthermore, the affinity of quinolones alone for the GABA_A receptors is actually relatively low, and to observe significant binding, experiments have to be conducted in the presence of a nonsteroidal anti-inflammatory derivative (NSAID), usually biphenyl acetic acid, the active metabolite of fenbufen (1, 16). Results of GABA binding experiments could therefore only be extrapolated, at best, to a situation in which quinolones are given together with an NSAID. Second, in vitro experiments, including new approaches such as the use of the Xenopus oocyte translation system of exogenous mRNA (9), do not take into consideration the pharmacokinetic characteristics of drugs, in particular, their ability to reach the receptors at the central level (4), which may vary considerably from one quinolone to another (7, 8).

To better characterize the convulsant activity of quinolones alone, we have previously used an experimental approach initially proposed to investigate the effect of disease states on the pharmacodynamics of drugs in vivo, alone (3) or in combination (12). The principle of this approach, which has since been used on many occasions, consists of measuring drug concentrations in the biophase (i.e., at the site of action) at the onset of activity (2). By using various drugs with hypnotic activity, including phenobarbital (2, 6), diazepam (10), and desmethyl-diazepam (11), or excitatory effects such as those caused by theophylline (13, 15, 17) or pentylenetetrazole (14), Levy and collaborators demonstrated that cerebrospinal fluid (CSF) was part of the biophase. Interestingly, we recently showed that at the onset of maximal seizures produced by two quinolones, pefloxacin and norfloxacin, CSF was also part of the biophase (3). Therefore, estimation of drug concentrations in the CSF at the onset of activity could be a very useful strategy to predict the potential epileptogenic risk of quinolones, especially because it does not require any hypothesis about the mechanism of action or the presence of an NSAID. However, these drug concentrations in CSF should be hardly estimated in humans and can only be obtained in laboratory animals. Therefore, information about the interspecies variability of drug concentrations in the CSF at the onset of maximal seizures is required before one can extrapolate the results obtained with animals to humans. That problem was addressed in this study.

A commercially available solution of pefloxacin methane sulfonate (Bellon Laboratories) titrating 240 mmol of pefloxacin per liter and a solution of norfloxacin hydrochloride (Sigma) dissolved in 5% glucose titrating 240 mmol/liter were used for this study (3). Animals were housed in the animal breeding facilities of our laboratory (authorization no. 0028). Swiss mice, Sprague-Dawley rats, and New Zealand rabbits (Depres Breeding Laboratories, St. Doulchard, France) were used in this study. All were male; their body weights (mean ± standard deviation [SD]) were, respectively, 27 ± 2, 264 ± 15, and 3,000 ± 431 g. Food was withdrawn 12 h before the experiment, but the animals had free access to water until drug infusion. Rats received intravenous doses of pefloxacin or norfloxacin through a cannula implanted in the left jugular vein 1 day before the experiment, as previously described (3). For mice and rabbits, the infusion was performed through a cannula (Microflex Infusion Set: 0.5 mm/G.25; Vygon Laboratories) immediately after its implantation in a tail vein for mice and in the marginal ear vein for rabbits. Infusion rates were equal to 260, 960, and 12,000 μmol/h for mice, rats, and rabbits, respectively (corresponding to 1, 4, and 50 ml/h). Administrations were conducted between 2:00 p.m. and 6:00 p.m. Each animal was kept under a heating lamp during infusion to maintain its body temperature. Rats could move freely during the whole duration of the infusion. Mice and rabbits, which had no implanted catheter, were initially kept restrained to ensure correct infusion, but when the first excitatory effects appeared, animals were allowed to move freely to improve...
TABLE 1. Parameters characteristic of the pharmacokinetic contribution of pefloxacin and norfloxacin to convulsant activity in mice, rats, and rabbits

<table>
<thead>
<tr>
<th>Drug and animals</th>
<th>Infusion time (min)</th>
<th>( C_u ) (µmol·liter(^{-1}))</th>
<th>( C_u ) (µmol·liter(^{-1}))</th>
<th>Dose/csf (liters·kg(^{-1}))</th>
<th>( C_u/C_u )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pefloxacin</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mice</td>
<td>9.8 ± 1.6</td>
<td>1,870 ± 870</td>
<td>1,627 ± 757</td>
<td>1.08 ± 0.45</td>
<td>0.18 ± 0.10</td>
</tr>
<tr>
<td>Rats</td>
<td>26.2 ± 1.6</td>
<td>590 ± 97</td>
<td>433 ± 64</td>
<td>3.71 ± 0.56</td>
<td>0.76 ± 0.13</td>
</tr>
<tr>
<td>Rabbits</td>
<td>12.4 ± 2.2</td>
<td>1,036 ± 253</td>
<td>625 ± 127</td>
<td>1.24 ± 0.30</td>
<td>0.25 ± 0.05</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mice</td>
<td>9.5 ± 1.2</td>
<td>1,685 ± 541</td>
<td>1,517 ± 487</td>
<td>1.14 ± 0.41</td>
<td>0.019 ± 0.006</td>
</tr>
<tr>
<td>Rats</td>
<td>26.3 ± 2.5</td>
<td>1,373 ± 378</td>
<td>1,173 ± 368</td>
<td>1.65 ± 0.41</td>
<td>0.044 ± 0.010</td>
</tr>
<tr>
<td>Rabbits</td>
<td>14.1 ± 2.0</td>
<td>1,732 ± 217</td>
<td>1,195 ± 150</td>
<td>0.85 ± 0.15</td>
<td>0.035 ± 0.011</td>
</tr>
</tbody>
</table>

\( *C_u\) total drug concentration in plasma.

\( \mu u\) Each value is the mean ± SD.

seizure activity observation. The volume of solution administered varied from 0.14 to 0.23 ml for mice, 1.6 to 2.0 ml for rats, and 8.8 to 14.5 ml for rabbits. Infusion times are reported in Table 1. Immediately after exhibiting maximal seizures, animals were anesthetized with an intramuscular injection of 50 mg of ketamine (KETALAR at 50 mg/ml; Parke Davis Laboratories) per kg and 20 mg of xylazine hydrochloride (ROMPUN; Bayer Laboratories) per kg and 20 mg of xylazine hydrochloride (ROMPUN; Bayer Laboratories) per kg, unless they had died following maximal seizures. CSF and blood were sampled within 3 and 5 min, respectively, following the end of the infusion. Clear CSF specimens were obtained by cisternal puncture, and blood was obtained from the abdominal aorta. Blood was collected in heparinized tubes and immediately centrifuged. Plasma was transferred into two separate tubes (rats and rabbits) or only one (mice). A fraction (rats and rabbits) or all (mice) of the plasma was kept frozen at −20°C until assayed for determination of the total drug concentration in plasma. The other fraction (rats and rabbits) was ultrafiltered with a Centrifree system (CF50A; Amicon) for determination of free drug concentrations (\( C_u \)). Because of the small plasma volume collected from mice, protein binding was estimated in this species by using spiked plasma at a concentration of 1.5 mmol/liter. Fluoroquinolone concentrations were determined by high-performance liquid chromatography as previously described (3). Briefly, separation was achieved with a Spherisorb octyldecylic silane column (5 µm; 300 by 4 mm [inside diameter]) and a mobile phase consisting of 0.1 M aqueous citric acid solution containing 13% (vol/vol) acetonitrile and 10 mM tetrabutyl ammonium perchlorate at a flow rate of 0.8 ml/min and detection by fluorimetry at an excitation wavelength of 280 nm without an emission filter (Spectroflow 980; ABI Analytical Kratos Division). The limit of quantification was on the order of 0.15 µmol/liter of plasma, plasma ultrafiltrate, and CSF for the two quinolones. The interday coefficients of variation calculated for each compound and estimated by adding known amounts of quinolones to blank plasma or a 0.9% NaCl solution (for the ultrafiltrate and CSF) at two concentration close to the usually measured values were equal to or less than 8%. The apparent permeability ratio was calculated from the ratio of the drug concentration in CSF (\( C_{sf}\)) to \( C_u\) at the onset of maximal seizures. Convulsant doses were calculated as the product of the infusion time multiplied by the rate of infusion, and the dose/\( C_u\) ratio was estimated. Results are expressed as means ± SD. Statistical comparisons were done by analysis of variance, followed by a Student t test, when appropriate, or by nonparametric analysis (Mann-Whitney test).

The pharmacodynamic contribution to the convulsant activity of pefloxacin and norfloxacin in the three animal species tested can be characterized by drug concentrations in CSF at the onset of activity (Fig. 1). Results obtained with rats were in generally good agreement with previously published data obtained by using a similar infusion rate of 960 µmol/h (3). Concentrations of norfloxacin in CSF at the onset of activity were virtually identical in these two studies (48.5 ± 5.9 versus 48.2 ± 11.9 µmol/liter [no significant difference]), and those of pefloxacin estimated in the present study were only 14% lower, on average, than those obtained in the initial study (323 ± 19 versus 377 ± 35 µmol/liter \( P < 0.01 \)). As a consequence of this difference, the ratio of pefloxacin to norfloxacin concentrations in CSF at the onset of maximal seizures was 6.6, compared to the 8.0 initially estimated (3). Concentrations of pefloxacin and norfloxacin in CSF at the onset of maximal seizures in a particular animal species were always significantly different \( (P < 0.001 ) \), and the concentrations of each quinolone in CSF were not exactly identical across species (Fig. 1). The lowest drug concentrations in CSF were measured in rabbits. They were only about half of the highest concentrations observed in rats, and these differences were statistically significant \( (P < 0.01) \). However, and more interestingly, the ratios of mean pefloxacin and norfloxacin concentrations in CSF were almost identical from one species to another at 7.0, 6.6, and 6.0 for mice, rats, and rabbits, respectively. This absence of major interspecies variability in the relative intrinsic convulsant activity of pefloxacin and norfloxacin in mice, rats, and rabbits suggests that such a six- to sevenfold difference in intrinsic convulsant activity could also exist in humans. Although this would evidently be very difficult to assess, determination of
concentrations of quinolones in CSF at the onset of maximal seizures in laboratory animals seems a useful way to compare the potential epileptogenic risk of quinolones in the early preclinical phase of development. At least this information should be gathered together with the more traditional in vitro binding data, which are notably also obtained with rats and therefore subject to interspecies extrapolation uncertainty.

The pharmacokinetic contribution to the convulsant activity of pefloxacin and norfloxacin in the three animal species tested can be characterized by the dose/$C_{\text{csf}}$ ratio or by two ratios, as presented in Table 1. The dose/$C_{\text{csf}}$ ratio, which is derived from units of liters per kilogram, reflects drug distribution in the whole body at the onset of activity, and $C_{\text{csf}}/C_{\text{u}}$ which has no units, reflects the ability of the drug to penetrate the CSF. Unfortunately, as opposed to the parameter characteristic of the pharmacodynamic contribution to the convulsant activity ($C_{\text{csf}}$ at the onset of activity), these ratios may also be affected by the duration of infusion. This explains why the convulsant dose may change with the infusion rate but not the drug concentration in the biophase, which is the rationale for using $C_{\text{csf}}$ rather than the dose to characterize the convulsant activity of quinolones (3). Therefore, these ratios, which are useful in understanding the apparent discrepancies observed between results at the CSF and dose levels (Fig. 1 and 2), must be interpreted very carefully, especially for interspecies comparisons.

In conclusion, determination of concentrations of quinolones in CSF at the onset of maximal seizures in laboratory animals provides early and unique information on the epileptogenic risk associated with the therapeutic use of these antibiotics. This information is of great value because it is the only way to estimate the pharmacodynamic contribution in vivo (i.e., the relationship between $C_{\text{csf}}$ and effect) to the convulsant activity of quinolones administered alone, with virtually no interspecies variability in the relative activities of different compounds, at least in mice, rats, and rabbits. Extrapolation of these data to the clinical situation in terms of convulsant doses rather than drug concentrations in CSF is then possible, providing the pharmacokinetic contribution to the convulsant activity is clearly understood. This new approach appears to be very useful; at least concentrations of quinolones in CSF at the onset of seizures should be used together with the traditional GABA binding experiments to predict the epileptogenic risk of quinolones at the early stage of development and to better define the relationships between the chemical structures and convulsant activities of these antibiotics.

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FIG. 2. Doses of pefloxacin (■) and norfloxacin (□) at the onset of maximum seizures in mice, rats, and rabbits. Each bar indicates the mean ± SD (error bars).

REFERENCES