Pharmacokinetics and pharmacodynamics of fluoroquinolones

George Drusano1, Marie-Thérèse Labro2, Otto Cars3, Paul Mendes4, Pramod Shah5, Fritz Sögel6 and Willi Weber7

1Division of Clinical Pharmacology, Department of Medicine, Albany Medical College, Albany, New York, USA; 2INSERM U479, Service d'Hematologie et d'Immunologie Biologiques, CHU Xavier BICHAT, Paris, France; 3Department of Infectious Diseases, Uppsala University Hospital, Uppsala, Sweden; 4Hoechst Marion Roussel Inc, Bridgewater, New Jersey, USA; 5Med Klinik III, Frankfurt am Main, Germany; 6Institute for Biomedical & Pharmaceutical Research, Nurnberg Herodsberg, Germany; 7Hoechst Marion Roussel AG, Clinical Development, Frankfurt am Main, Germany

INTRODUCTION

Since 1962, when the first quinolone derivative with antibacterial activity, nalidixic acid, was synthesized, there has been a concerted and systematic search for compounds with enhanced antibacterial activity and/or an improved pharmacokinetic profile. The potent activity of nalidixic acid against Gram-negative bacteria has been greatly improved and expanded upon by combining a 7-piperazinyl ring and a 6-fluorine atom on the quinolone nucleus (as in the case of ciprofloxacin, norfloxacin and pefloxacin) or on the 1,8-naphthyridine nucleus (as in enoxacin), or producing fluorinated tricyclic derivatives (levofloxacin and ofloxacin). The fluoroquinolones can be divided into four groups on the basis of their chemical structure—monocyclic, bicyclic, tricyclic and tetracyclic structures linked to the pyridone β-carboxyl acid nucleus—or into four biological groups according to their antibacterial spectrum and extent of metabolic transformation. The earlier quinolone derivatives have a limited antibacterial spectrum and are active mainly against the Enterobacteriaceae. These compounds are characterized by unfavorable pharmacokinetic properties and low in vivo potency. The newer derivatives have more favorable pharmacokinetic profiles, resulting in higher serum concentrations, and have lower minimum inhibitory concentrations (MICs), making them appropriate for the treatment of systemic infections [1]. This paper focuses on the tissue penetration, intracellular accumulation and pharmacodynamics of the fluoroquinolones.

TISSUE PENETRATION

It is essential that antibacterial agents used to treat infections caused by intracellular bacteria have favorable intracellular penetration, accumulation and disposition. Concentrations of antibiotics in various tissues have attracted considerable interest. The fluoroquinolones are able to achieve high concentrations in cells, although the exact mechanism by which this occurs is not known. Lipophilicity does not seem to play a significant role, since some of them are hydrophilic [2].

As with other antibiotics which exhibit intracellular accumulation (e.g. tetracyclines, macrolides), the concentrations of fluoroquinolones in different tissues are often higher than the concurrent serum levels. One must, however, bear in mind that tissue concentrations are difficult to interpret. Since drugs are not distributed evenly throughout the body or within a specific tissue, concentrations obtained by

Corresponding author and reprint requests:
George Drusano, Division of Clinical Pharmacology, Department of Medicine, Albany Medical College, Albany, NY 12208, USA
Tel: +1 518 262 6761 Fax: +1 518 262 6333
E-mail:
homogenizing tissue are inherently inaccurate and, as these are mean values of concentrations in different tissue compartments, they cannot be used to estimate the drug concentration at the site of action [3, 4]. This is clearly evident from the studies of Baldwin et al, where concentrations of ciprofloxacin and lomefloxacin in bronchial biopsies, alveolar epithelial lining fluid and alveolar macrophages were compared with simultaneous serum levels [5]. The bronchial biopsy concentrations of ciprofloxacin and lomefloxacin were 1.6 and 1.7 times those of serum, respectively. The corresponding values for macrophage concentrations were 11.8 and 20.1, respectively. Interestingly, the concentrations of the two fluoroquinolones in epithelial lining fluid were also higher than in serum (2.1 and 1.9 times the serum levels, respectively), possibly indicating antibiotic delivery to the extracellular site from the intracellular antibiotic reservoir. Similar results have been obtained with levofloxacin which showed higher concentrations in epithelial lining fluid and alveolar macrophages than in serum [6].

The common bacteria causing acute respiratory tract infections are extracellular pathogens. The bactericidal action of the antibiotic is thus normally required in the interstitial fluid. In most tissues, the antibiotic molecules diffuse freely between the vascular and interstitial fluid space. Even if serum and interstitial fluid levels of antibiotics do not always equilibrate, such as in large collections of inflammatory fluid, the concentrations of free (non-protein-bound) antibiotic are the most useful surrogate markers for the concentrations achieved at the site of infection for extracellular pathogens. However, for respiratory tract infections caused by intracellular bacteria, such as Legionella spp. and Chlamydia pneumoniae, the high intracellular levels of fluoroquinolones may be important.

**Intracellular accumulation of fluoroquinolones**

The cellular pharmacokinetics of antibacterial agents is one of the parameters responsible for their intracellular activity (Figure 1). Fluoroquinolones are able to concentrate within phagocytic and non-phagocytic cells and remain active against different facultative, obligate, intracellular pathogens. Many studies have dealt with the characteristics of the cellular accumulation of fluoroquinolones in vitro, particularly in phagocytic cells.

The classical technique used to determine the various parameters of cellular pharmacokinetics (accumulation kinetics, cellular location and efflux) involves in vitro incubation of the test cells with the drug (either radiolabeled or unlabeled) followed by velocity centrifugation through a water-impermeable oil cushion to separate the free antibiotic (aqueous phase) from the

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**Figure 1** Parameters of antibacterial bioactivity.
cell-associated drug. Drug measurement may be done either by a fluorometric assay, owing to the fluorescent property of most fluoroquinolones, or by radioactivity counting. The amount of cell-associated drug (ng/mg cells or ng/mg protein) is determined by standard drug dilutions. After determination of the intracellular water content (by isotopic techniques, cytometry, etc.), it is possible to calculate the intracellular concentration (mg/L) and, from that, the parameter most frequently used for comparison, the cellular/extracellular concentration ratio (C/E).

It is important to determine the location of the drug (by cell fragmentation techniques), e.g. in cytosol, granules or lysosomes, to ensure that the C/E ratio represents the true ratio between intra- and extracellular concentration. Otherwise, the C/E ratio may just characterize the overall amount of cell-associated drug, including the membranes and associated ‘true’ intracellular compartments. Drug efflux is measured by similar techniques, using drug-loaded cells isolated and placed in drug-free medium. At repeated time intervals, aliquots are centrifuged and the drug measured in both the supernatant and the cell pellet to calculate the percentage of drug released. Variations in the experimental medium (such as pH and inhibitors) and other conditions (such as temperature and killed cells) are used to investigate the mechanisms underlying drug uptake and efflux.

The main features of fluoroquinolone cellular pharmacokinetics are shown in Table 1 [7–16]. The

### Table 1 Comparative cellular pharmacokinetics of fluoroquinolones

<table>
<thead>
<tr>
<th>Drug (technique)</th>
<th>Cell</th>
<th>C/E ratio (time)</th>
<th>Efflux (time)</th>
<th>Activity (bacteria)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>MPH (m)</td>
<td>2.7 (30 min)</td>
<td>ND</td>
<td>+ (S.a.)</td>
<td>7</td>
</tr>
<tr>
<td>(bioassay)</td>
<td>PMN</td>
<td>4 (30 min to 2 h) to 8 (5 min)</td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>(HPLC)</td>
<td>PMN</td>
<td>4.7 (20 min)</td>
<td>30% (5 min)</td>
<td>+ (P.a.)</td>
<td>9</td>
</tr>
<tr>
<td>(fluorometry)</td>
<td>J774</td>
<td>4.4 (5 min)</td>
<td>+ (S.a.)</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Levofoxacin</td>
<td>PMN</td>
<td>6.1 (20 min)</td>
<td>&gt;8.3 (10 min)</td>
<td>+ (S.a.)</td>
<td>9</td>
</tr>
<tr>
<td>(fluorometry)</td>
<td>Hep 2</td>
<td>2.1 (10 min)</td>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>McCoy</td>
<td>3.9 (10 min)</td>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Vero</td>
<td>2.1 (10 min)</td>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>PMN</td>
<td>4.9 (5 min) to 7.6 (30 min)</td>
<td>65–88% (5 min)</td>
<td>+ (P.a., S.a.)</td>
<td>9 (10)</td>
</tr>
<tr>
<td>(fluorometry)</td>
<td>Hep 2</td>
<td>2.8 (10 min)</td>
<td></td>
<td></td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>McCoy</td>
<td>4.7 (10 min)</td>
<td></td>
<td></td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Vero</td>
<td>2.4 (10 min)</td>
<td></td>
<td></td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>J774</td>
<td>5.3 (5 min)</td>
<td>+ (S.a.)</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>(HPLC)</td>
<td>WI-38</td>
<td>8.6 (30 min)</td>
<td>+ (S.e.)</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Sparfloxacin</td>
<td>PMN</td>
<td>6.5 (20 min)</td>
<td>60% (5 min)</td>
<td>+ (S.a.)</td>
<td>9</td>
</tr>
<tr>
<td>(fluorometry)</td>
<td>Hep 2</td>
<td>7 (20 min)</td>
<td></td>
<td></td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>McCoy</td>
<td>10 (20 min)</td>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>J774</td>
<td>6.4 (30 min)</td>
<td>+ (S.a.)</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Trovafloxacin</td>
<td>PMN</td>
<td>11 (20 min)</td>
<td>80% (5 min)</td>
<td>+ (S.a.)</td>
<td>16</td>
</tr>
<tr>
<td>(radioactivity)</td>
<td>MPH (m)</td>
<td>10 (20 min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>McCoy</td>
<td>9.6 (20 min)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

h, human; m, mouse; MPH, macrophage; ND, not determined; P.a., Pseudomonas aeruginosa; PMN, polymorphonuclear neutrophil; S.a., Staphylococcus aureus; S.e., Salmonella enteridis.
uptake of most fluoroquinolones is rapid, followed by a long-lasting plateau. Whatever the cell species, the C/E ratio is usually moderate (i.e. <10) [17]. The cellular location, mainly cytosolic, has been determined only for fleroxacin, lomefloxacin and pefloxacin [18]. Efflux is also rapid, with 60–90% of the intracellular drug being released within the first 5 min.

The mechanism underlying the accumulation of fluoroquinolones has not been clarified. Fluoroquinolone uptake does not appear to be saturable; most studies demonstrate that decreasing the incubation temperature to 4°C results in decreased uptake except for trovafloxacin, which shows increased uptake at 4°C (C/E ratio of 62 at 4°C versus 11 at 37°C). and sparfloxacin, which is taken up by neutrophils to a similar extent at 4°C and 37°C [10–13,15–18]. Uptake of ofloxacin and ciprofloxacin is not affected by external pH, whereas uptake of trovafloxacin and pefloxacin is increased, and that of sparfloxacin is decreased at pH 8 [10,13,15,16,18]. Metabolic inhibitors do not seem to modify drug uptake; only the high concentration of 5 mM NaCN impairs ofloxacin, levofloxacin and ciprofloxacin uptake [10]. Various competitive inhibitors (e.g. hexose, nucleosides and amino acids of known membrane transfer systems) do not impair fluoroquinolone uptake. Inhibition of ofloxacin uptake by amino acids was previously reported by Pascual et al., but other authors did not confirm the results of this study [10,13,17].

All of these studies reinforce the hypothesis that passive diffusion mechanisms are involved in fluoroquinolone uptake and that the physicochemical properties of these drugs (e.g. ionization, lipophilicity) may explain the differences observed in cellular accumulation. It has been suggested that the efflux mechanism of fluoroquinolones depends on pro-benecid (gentamycin)-inhibitable organic anion transport. To date, only norfloxacin, tetracyclaxacin and ciprofloxacin have been assessed in this context [19,20].

The main consequence of the intracellular accumulation of fluoroquinolones concerns their intracellular activity. Many pathogens which reside in phagolysosomes (e.g. Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacteriaceae) are susceptible to the bactericidal action of fluoroquinolones in vitro (Figure 1). It seems that the intrinsic bactericidal mechanism of phagocytes (particularly oxidant production) is necessary for optimal activity [21]. For intracellular pathogens located in phagosomes (e.g. Legionella pneumophila, Mycobacteria), many reports show that fluoroquinolones are also effective [22,23]. The cellular accumulation of pefloxacin was enhanced in Legionella-infected mouse peritoneal macrophages compared to non-infected cells, whereas its efflux was decreased, and the drug was located in the same compartment as the bacteria [18]. However, the mechanisms still remain unknown.

In vivo, in experimental models and various infectious diseases, fluoroquinolones have proved effective against infections caused by intracellular pathogens. Since they are frequently used in immunocompromised individuals or as combination therapy for mixed infections, it is interesting to note that their cellular uptake (at least in vitro) is not modified by various antimicrobial agents and chemotherapeutic drugs [9,24,25]. Targeted delivery of fluoroquinolones by drug-loaded phagocytes (e.g. polymorphonuclear neutrophils (PMNs)) has been suggested but no data are available. Modification of cell functions, particularly cytokine production and lymphocyte activation, has been observed in vitro, usually with high, non-therapeutic concentrations of these drugs. Further studies are required to obtain the data.

**PHARMACOKINETICS IN HUMANS**

The absolute bioavailability of selected fluoroquinolones is shown in Table 2 [26]. Ofloxacin, levofloxacin, pefloxacin, fleroxacin, tetracyclaxacin and lomefloxacin are almost completely absorbed, while ciprofloxacin and enoxacin have a lower bioavailability. The absorption of N6-methylated compounds, such as ofloxacin, levofloxacin, pefloxacin and fleroxacin, tends to be greater than that of non-N6-methylated compounds. At the acidic pH of the stomach, all fluoroquinolones are protonated and exist as the cationic species. Although the solubility of most fluoroquinolones is still not high at the slightly alkaline pH of the jejunum, they are well absorbed in the duodenum and jejunum, possibly because the percentage of the more lipophilic neutral species of the agent increases. The critical role of dissolved drug suggests that the absorption kinetics of fluoroquinolones may be affected to a great degree by their solubility and dissolution rates. This is supported by evidence that the T_max of tetracyclaxacin formulations increases with dose [26]. After oral administration, both the maximum serum concentration (C_max) and time to reach this concentration (T_max) are affected primarily by the absorption constant.

Little is known about the mechanism of absorption. Lipophilicity seems to govern the time course of absorption and is a function of the absorption coefficient k_s. Absorption of ciprofloxacin, pefloxacin and probably other fluoroquinolones is not affected by age, presence of cystic fibrosis, or renal dysfunction [26]. Studies in human volunteers suggest that absorption is passive. The fluoroquinolones most prone to the effects of food on absorption are ciprofloxacin
Table 2 Pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Drug</th>
<th>F (%)</th>
<th>Cl (mL/min)</th>
<th>Clr (mL/min)</th>
<th>Clark (mL/min)</th>
<th>Vd/F (L/kg)</th>
<th>f, (of dose)</th>
<th>fmax, (of dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>60-70</td>
<td>550-750</td>
<td>300-400</td>
<td>280-320</td>
<td>25-35</td>
<td>≤65</td>
<td>15.2</td>
</tr>
<tr>
<td>Olofoxacin</td>
<td>NA</td>
<td>41.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.3</td>
<td>3.7</td>
<td>97.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.6</td>
<td>NA</td>
</tr>
<tr>
<td>Enoxacin</td>
<td>70-90</td>
<td>500-650</td>
<td>260-350</td>
<td>220-300</td>
<td>2.5-3.0</td>
<td>≤60</td>
<td>NA</td>
</tr>
<tr>
<td>Fleroxacin</td>
<td>95-100</td>
<td>100-120</td>
<td>70-90</td>
<td>25-40</td>
<td>0.8-1.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>60-75</td>
<td>15</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>100</td>
<td>140-200</td>
<td>100-140</td>
<td>40-60</td>
<td>1.1-1.3</td>
<td>60-90</td>
<td>12-15</td>
</tr>
<tr>
<td>Lomefoxacin</td>
<td>95-100</td>
<td>200-250</td>
<td>180</td>
<td>70</td>
<td>1.5-2.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>70</td>
<td>NA</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>&lt;35&lt;sup&gt;f&lt;/sup&gt;</td>
<td>NA</td>
<td>250</td>
<td>NA</td>
<td>NA</td>
<td>&lt;30</td>
<td>NA</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>95-100</td>
<td>200-250</td>
<td>160-200</td>
<td>40-50</td>
<td>1.2-1.4</td>
<td>80-90</td>
<td>NA</td>
</tr>
<tr>
<td>Pefloxacin</td>
<td>95-100</td>
<td>140-130</td>
<td>7-12</td>
<td>100-120</td>
<td>1.1-1.7</td>
<td>6-10</td>
<td>60&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sparfloxacin</td>
<td>60&lt;sup&gt;h&lt;/sup&gt;</td>
<td>160-200&lt;sup&gt;i&lt;/sup&gt;</td>
<td>15-20</td>
<td>130-170</td>
<td>1.6-1.9&lt;sup&gt;j&lt;/sup&gt;</td>
<td>35-50</td>
<td>NA</td>
</tr>
<tr>
<td>Temafloxacin</td>
<td>~95&lt;sup&gt;k&lt;/sup&gt;</td>
<td>100-120</td>
<td>120-160</td>
<td>60-90</td>
<td>1.7-2.1</td>
<td>60-70</td>
<td>70</td>
</tr>
<tr>
<td>Tosufloxacin</td>
<td>&lt;30&lt;sup&gt;l&lt;/sup&gt;</td>
<td>NA</td>
<td>90-120</td>
<td>NA</td>
<td>NA</td>
<td>≤40&lt;sup&gt;l&lt;/sup&gt;</td>
<td>NA</td>
</tr>
</tbody>
</table>

Adapted with permission from the American Journal of Medicine [26].

F = fraction of dose absorbed; Cl = total clearance; Clr = renal clearance; Clark = non-renal clearance; Vd/F = volume of distribution; f, = fraction of dose excreted unchanged in urine; fmax, = fraction of dose excreted unchanged in feces; NA = data not available.

<sup>a</sup> Excreted as unchanged compound.

<sup>b</sup> CI/F.

<sup>c</sup> Vd/F.

<sup>d</sup> Vd,(Vd at steady state).

<sup>e</sup> Percentage may be smaller, since no intravenous dosage can be studied.

<sup>f</sup> Only data on excretion of total 14C radioactivity were used; no discrimination was made between parent drug and metabolite.

<sup>g</sup> Numbers are estimates of the minimal dose absorbed. The actual number may be substantially higher.

<sup>h</sup> Dependent on food intake (without food <30; with food ≤40).

and norfloxacin [26]. The extent of absorption of norfloxacin may also be affected, but that of ciprofloxacin is not [26]. Total absorption of ofloxacin and levofloxacin is unaltered when given with normal or fat-rich food [26]. An unchanged AUC for fleroxacin, ofloxacin, levofloxacin, pefloxacin, and norfloxacin suggests that N\textsuperscript{6}O\textsubscript{1}-methylation protects against the effects of food. Food does not alter the overall absorption but prolongs the T\textsubscript{max} slightly but significantly [26]. Delay in gastric emptying may cause the increase in T\textsubscript{max} of fluoroquinolones ingested with fat-rich food.

Elevated gastric pH may enhance or delay absorption by affecting dissolution and may shift the equilibrium between ionized (less absorbed) and non-ionized (better absorbed) forms. Antacids may increase gastric emptying time and thus reduce the C\textsubscript{max} and delay the T\textsubscript{max}. The chelating effect of antacids on absorption depends on the interval between fluoroquinolone and antacid intake. Therefore, the dose of antacid should not be given earlier than 2 h after the fluoroquinolone dose.

Most fluoroquinolones are excreted primarily by the kidney but the gastrointestinal route also plays a significant role in the overall elimination of these agents [26]. Hepatic elimination plays a role only for certain agents such as pefloxacin [26]. The physicochemical properties of fluoroquinolones are greatly influenced by their two functional groups, the carboxyl group and basic group, which affect pH-dependent lipophilicity. Many important properties of distribution may be explained by interactions between the pH of these groups and the local pH in the body. These physicochemical properties may also partly explain the elimination properties of fluoroquinolones. They all undergo tubular secretion as either acids or bases and some are also significantly reabsorbed. Hepatic handling and resultant metabolite excretion is also influenced by N\textsuperscript{6}O\textsubscript{1}-methylation.

PHARMACODYNAMICS

Pharmacodynamics deals with the relationship between exposure to a drug and a resulting effect, either therapeutic or toxic. Through delineation of such exposure–response relationships, therapy for the patient can be optimized and toxicity minimized while the therapeutic response is maximized. There have been many studies of anti-infective agents in which drugs of different classes have had their pharmacodynamics determined for bacteria, as well as for other pathogens. For the fluoroquinolones in particular, a considerable amount of pharmacodynamic data has been recorded. These data stem from in vitro studies, animal models of infection and clinical studies. In the case of bactericidal agents, the rate of kill can be thought of as the pharmacodynamic variable most closely linked to
organism kill at the primary infection site. To gain an insight into bacterial killing dynamics, two pieces of information are critical. The first is a measure of drug exposure and the second is a measure of drug potency for the pathogen in question.

Multiple measures of drug exposure can be posited. The first issue concerns whether drug concentrations should be directly measured at the primary infection site or whether serum exposure measures will suffice. In the majority of cases, the pharmacokinetics are linear. Therefore, first-order rate constants will link drug transfer from serum to the site of infection. Consequently, while the actual concentration-time profiles will differ slightly between two sites, linkage to the effect will not be impeded, as different parameters will be obtained for whatever function is employed for the analysis. Consequently, either central or peripheral compartment concentrations may be used. For case of data collection, this will usually result in serum data being chosen for the pharmacodynamic analysis.

Reducing the number of pathogens located at the site of infection is the primary target of an antibiotic. Only the drug concentration at the site of infection is relevant for its degree of efficacy. However, it is not possible to determine the exact location of the infection where the concentration of interest should be measured. In general, therefore, the serum concentration is used as a surrogate marker of the infection compartment concentration. After administration of an antibiotic, the infection site concentration will follow the changes in serum concentration. This delay is characterized by an equilibration half-time. When pharmacokinetics are linear and a steady-state situation is established, the infection site concentration is directly proportional to the serum concentration, which reflects exposure of the pathogens to the drug and, therefore, should be a good surrogate marker (Figure 2).

For situations where different organisms will be part of the analysis (as in the analysis of clinical trial data), it is important to normalize the drug exposure to the measure of potency of the drug for the pathogen (in most instances, this will be the MIC). This results in three pharmacodynamic variables which are drawn from various aspects of the shape of the concentration-time curve: the peak/MIC ratio, the AUC/MIC ratio (AUC) and the time>MIC, which may all be linked to outcome. If the peak/MIC ratio is linked to outcome, this indicates that relatively infrequent dosing intervals would lead to the best results, with production of a very peaked shape for the concentration-time curve. The AUC/MIC ratio would indicate that the therapeutic outcome is independent of curve shape, and time>MIC would mean that flat curve shapes which keep trough levels high would produce the best results.

Fluoroquinolones not only have activity against extracellular pathogens but are also active against intracellular pathogens such as *Mycoplasma* and *Chlamydia*, amongst others. Their ability to enter cells is probably the key to their activity against these pathogens. Generally, they penetrate well into most cellular compartments where these organisms reside. In addition, the uptake into cells may explain some of the findings of the concentration-time curve in the lung for fluoroquinolones when measured by the technique described by Baldwin et al [27]. Concentrations of fluoroquinolones in extracellular fluid often considerably exceed those seen in the serum. A partial explanation for this is efflux of drug from lung cells and lung macrophages, which concentrates fluoroquinolones intracellularly because of ion-trapping effects. When serum concentrations decline, this could allow efflux of drug from cellular reservoirs which would maintain drug concentrations locally in excess of the serum levels.

The concentration dependence of the bacterial kill rate will determine which of these dynamic variables is most closely linked to organism kill. If the drug is not very concentration-dependent in kill rate (as is the case with β-lactam agents), the rate of kill engendered by high concentrations around the peak will not differ substantially from the kill rate engendered by concentrations down near the MIC. Consequently, the number of organisms killed by a dose of drug will be relatively constant, i.e. the kill rate multiplied by the time that the concentration exceeds the MIC. In this
case, time>MIC would be the pharmacodynamic variable of interest.

For drugs which are very concentration-dependent in kill rate (e.g. fluoroquinolones and aminoglycosides), the kill rate changes continuously with concentration, and the total number of organisms killed is provided by the path integral of kill rate (which is concentration-dependent) over time. That is, the kill rate near the peak multiplied by the duration over which it is appropriate (i.e. until the concentration is reached which produces a noticeably changed kill rate) plus the next kill rate multiplied by the time over which it is effective, and so on. With this situation, the AUC/MIC ratio should be most closely linked to outcome, as the AUC is, itself, the integral of the concentration-time curve.

The peak/MIC ratio should never be linked to outcome in the scenario created. Were this so, there would be an identifiable dose of drug which had demonstrable activity, whereas half this dose would have markedly reduced activity to almost nil. In general, antibiotics do not behave in this manner because the scenario, as generated, is incomplete and examines the sensitivity of the organism to the drug as a static phenomenon. However, it is known that dense bacterial populations, such as are seen in serious infections, are composed of distributions of organisms, some of which may be spontaneously mutated to altered drug susceptibility. Frequently, there will be a number of resistant mutants in large populations of organisms. In the case of nosocomial pneumonia, where Enterobacter cloaceae and P. aeruginosa are common pathogens, bacterial densities are high and there is considerable pus in the chest, giving a total population of 10^10–10^11. Mutational frequencies for resistance differ, and for these pathogens, mutational frequencies are in the order of 10^5–10^6 and 10^6–10^7, respectively. Consequently, there are 10^4–10^5 resistant organisms present before any selective pressure (antibiotic administration) has been applied.

With fluoroquinolones, resistant Gram-negative organisms are frequently mutated in the gyrA gene, and Gram-positive bacteria in the parC gene, with single point mutations most frequently causing a four- to eight-fold shift in the MIC. The size of the MIC change is important, because larger changes (normally 16- to 128-fold), as are seen with the selection of mutants stably derepressed for Bush class I enzyme production by some B-lactams, could not be counter-selected with a greater dosing intensity. It may be that peak/MIC plays a role here. If a high enough peak/MIC ratio can be achieved, not only may the parent organism be killed, but also the gyrA mutant (or any other type of mutant with a relatively low change in MIC) may be suppressed. Consequently, while AUC/MIC can be thought of as being linked to cell kill for the parent strain, for peak/MIC suppression of resistant subpopulations should be considered as well.

In vitro data
Studies which have shed considerable light on fluoroquinolone pharmacodynamics are those by Blaser et al and Dudley et al, employing the hollow-fiber pharmacodynamic model [28,29]. This model is particularly instructive, as human serum pharmacokinetic-time profiles can be simulated and effects on organism load ascertained. Blaser et al examined enoxacin against a strain of Klebsiella pneumoniae [28]. In a series of experiments, once-daily dosing with enoxacin was compared with half the dose administered twice daily (same total daily dose). In one of three experiments with twice-daily dosing, there was clearcut breakthrough growth which was not seen with once-daily dosing (Figure 3) [28]. They then performed a large number of experiments and plotted residual organisms determined at the end of the experiment. As can be seen in Figure 4 [28], when an organism–dosing regimen pair had a resultant peak/MIC ratio of 10/1 or greater, the unit was always completely sterilized. This is important, as it lends credence to the idea of suppressing resistant mutants by employment of specific dosing regimens.

This idea was directly addressed by Dudley et al with P. aeruginosa [29]. They simulated an intravenous dose of ciprofloxacin 200 mg on a 12-hourly schedule (a regimen then in frequent clinical use). The baseline bacterial population was examined on agar plates containing differing concentrations of ciprofloxacin. This was then repeated prior to the 12-h simulated dose and again at 24 h. The administration of ciprofloxacin at a suboptimal dose rapidly selected the resistant part of the initial bacterial population (Figure 5) [29]. As well as amplifying the resistant mutant subpopulation, this model enables the effects of altering dose and schedule on bacterial cell kill to be seen. It also provided the first clue that dosing could be modified to obtain a specific end: suppression of the resistant part of the bacterial population.

Animal models
Leggett et al and Fantin et al provided considerable insight into the pharmacodynamics of fluoroquinolones in an animal infection model [30,31]. This was the standardized neutropenic mouse thigh infection model employed by Craig's laboratory in which mice are made neutropenic with cyclophosphamide [32]. The pathogen is injected into the mouse thigh and allowed to multiply for several hours. Antimicrobial
Figure 3 Bactericidal activity of two enoxacin regimens against *Klebsiella pneumoniae* in a pharmacokinetic model. Cultures were eradicated in three of three experiments when the total daily dose was given as one single dose but in only two of three experiments when the same total daily dose was given as two equal doses [28]. Reproduced with permission from *Antimicrobial Agents and Chemotherapy*.

Figure 4 Antibacterial effect of multiple-dose regimens of enoxacin and netilmicin against five organisms. Changes in bacterial numbers during treatment periods of 4 and 24 h are plotted against the ratios of peak concentration to MIC. $t = \text{time}$ [28]. Reproduced with permission from *Antimicrobial Agents and Chemotherapy*. 
regimens are then initiated and the mice sacrificed at various time points, and the number of organisms in the thigh determined. Large numbers of doses and schedules are examined and the linkage of one of the three above mentioned pharmacodynamic variables determined by multivariate regression analysis. For fluoroquinolones, it was clear that the AUC/MIC ratio was the dynamic variable most clearly linked to outcome. This supports the theory that drugs which are concentration-dependent in kill rate will have an AUC/MIC ratio linked to outcome. However, it seems to contradict the data of Blaser et al and Dudley et al [28,29].

Some insight into this discrepancy was provided by Drusano et al employing a neutropenic rat model first described by Johnson [33,34]. Rats are made profoundly neutropenic with cyclophosphamide and then challenged with a dense inoculum intraperitoneally. As in the Craig model, the organisms are allowed to multiply before therapy is initiated. However, in this model, there is very reliable bacteremia and survival is used as the endpoint. An important point to emphasize is that the inoculum in the Craig model is of the order of $10^9$ microorganisms, while in the Johnson model (at least in this series of experiments) an inoculum of $10^9$ *P. aeruginosa* bacteria ($100 \times LD_{50}$) was employed. As might be expected, there is a relatively low probability that a resistant mutant will be included in the inoculum in the Craig model, whereas the larger inoculum in the Johnson model provides a much higher probability of having multiple resistant mutants in the population (mutational frequency for fluoroquinolone resistance in *Pseudomonas* spp. is frequently in the range of $10^{-6}$ to $10^{-7}$).

**Figure 5** Bactericidal activity of ciprofloxacin and subpopulation analysis of *Pseudomonas aeruginosa* 810 in an in vivo model following two simulated 200-mg doses. Values are mean ± SD [29]. Reproduced with permission from the American Journal of Medicine.
A series of dose-finding experiments identified 80 mg/kg of lomefloxacin as the dose which would generate approximately 70% survival in this model [33]. This is still on the relatively steep part of the exposure–response curve. An experiment was then performed with 200 animals (50 animals per group). One group received saline placebo, while the other three all received 80 mg/kg per day, but on three different schedules of administration (80 mg/kg o.d., 40 mg/kg b.i.d. and 20 mg/kg q.i.d.). A pharmacokinetic study documented that the AUCs were not statistically different for the three regimens and, thus, all had the same AUC/MIC ratio. As expected, the o.d. dosing group had the highest peak/MIC ratio, while the 20 mg/kg four times a day group had the longest time>MIC. If AUC/MIC were linked to outcome, all three regimens should provide the same outcome. If time>MIC were linked to outcome, four times a day dosing should be best, and if peak/MIC ratio is the linked dynamic variable, o.d. dosing should provide the greatest survival. The results are shown in Figure 6 [33].

Two things are evident. The first is that o.d. dosing provided a significantly better outcome ($p<0.001$). The second is that the survival curves for the other groups are, in essence, identical. This caused us to hypothesize that, when peak/MIC ratio does not exceed 10:1 (as it did not in the 40 mg/kg twice a day and 20 mg/kg four times a day groups), the dynamically linked variable is the AUC/MIC ratio. When this ratio is achieved, the peak/MIC ratio explains outcome better, most likely for the reasons evident in the Blaser et al and Dudley et al in vitro experiments, i.e. suppression of emergence of resistance [28,29]. The second experiment examined three dosing groups of 20 animals each (control, 40 mg/kg o.d. and 20 mg/kg twice a day). The results are shown in Figure 7 [33]. Clearly, when none of the regimens could produce a peak/MIC ratio of >10:1, AUC/MIC was seen to be linked to outcome (no difference between o.d. and twice a day dosing).

The experiments above examined the issue of response with changing drug exposure, with the MIC constant. The third set of experiments addressed holding the exposure constant and examining outcome as a function of changing MIC. Two mutant organisms were selected and found to have stable MICs of four and eight times that of the parent strain (MIC of 1, 4 and 8 mg/L). Consequently, challenges were used with the same genetic background and virulence, but differing in MIC. Four dosing groups (plus three control groups not displayed) were examined. A regimen of 80 mg/kg o.d. was used for each of the three organisms with the different MICs, producing both peak/MIC and AUC/MIC ratios for the three

![Figure 6](https://example.com/figure6.png)  
Figure 6: Dose fractionation experiment 1. The MIC of lomefloxacin for the challenge organism was 1 mg/L. There were 50 animals evaluated per group [33]. Reproduced with permission from Antimicrobial Agents and Chemotherapy.
groups in ratios of 8:4:1. The fourth group employed a dose of 20 mg/kg with the MIC=1 mg/L challenge organism, producing peak/MIC and AUC/MIC ratios equivalent to the group with 80 mg/kg o.d. dosing and a MIC=4 mg/L challenge organism. The results are shown in Figure 8 [33].

Changing organism MIC had, as expected, a profound effect on the outcome seen. Most importantly, outcomes were essentially identical for the two groups with identical AUC/MIC and peak/MIC ratios. This indicates that it does not matter whether the MIC (a more or less sensitive organism is being treated) or the exposure (larger or smaller doses are employed for treatment) are changed; the outcome is driven by the ratio of exposure to MIC (peak/MIC or AUC/MIC), however it is achieved.

**Clinical studies**

The first clinical data regarding the pharmacodynamics of fluoroquinolones were published by Peloquin et al [35]. They examined ciprofloxacin in a study of patients with lower respiratory tract infection caused by Gram-negative bacilli and concluded that time>MIC was the pharmacodynamic variable most closely linked to outcome. This somewhat discordant result was modified by their finding that the emergence of resistance among *P aeruginosa* was, indeed, driven by having a peak/MIC ratio which did not exceed 10:1.

Further patients were studied and the data reanalyzed by Forrest et al [36,37]. Patients were classified as having succeeded or failed clinically and microbiologically but, importantly, the lower respiratory tract flora were sampled daily and the time to initial pathogen eradication determined. The first two endpoints were subjected to logistic regression analysis, and the time to eradication endpoint was examined by Cox proportional hazards modeling.

The logistic regression analysis produced statistically significant linkages between the AUC/MIC ratio and both the probability of clinical success and that of microbiological success. It should be noted, however, that the authors also indicated that the correlation between AUC/MIC ratio and peak/MIC ratio in their population was 0.9. The Cox proportional hazards modeling again showed a significant linkage between AUC/MIC ratio and the time to bacterial eradication. Indeed, they also showed differences by AUC/MIC thresholds. A patient with an AUC/MIC ratio ≥125 had significantly shorter times to eradication than a patient with a smaller AUC/MIC ratio. Further, a patient with an AUC/MIC ratio ≥250 had a time to eradication significantly shorter than a patient with a ratio between 125 and 249 (Figure 9) [37]. These data are an elegant validation of the in vitro and animal model data. However, it should be recognized that the analysis was retrospective. Further, it should be empha-
Figure 8 Effect of altered MIC upon survivorship. Three isogenic organisms for which the lomefloxacin MICs were different served as the bacterial challenge. The three groups (MICs of 1, 4 and 8 mg/L) received 80 mg/kg every 24 h. A fourth group (○) had the strain for which the MIC was 1 mg/L used as the challenge organism and a dosing regimen of 20 mg/kg every 24 h. This provided the same peak/MIC ratio as the challenge organism for which the MIC was 4 mg/L used with animals treated with 80 mg/kg every 24 h. There were saline-treated controls for each challenge organism (all died). Twenty animals were evaluated [33]. Reproduced with permission from Antimicrobial Agents and Chemotherapy.

Figure 9 Time (days of therapy) to bacterial eradication versus AUIC (AUC/MIC ratio) illustrated by a time-to-event (survival) plot. Therapy versus the percentage of patients remaining culture positive on that day is shown. The three AUIC groups differed significantly (p<0.005) [37]. Reproduced with permission from Antimicrobial Agents and Chemotherapy.
sized that the outcome is specific to the population from which the patients were drawn: mainly elderly patients with very severe hospital-acquired lower respiratory tract infection (the majority due to Gram-negative pathogens) on mechanical ventilation. The findings should not be extrapolated beyond this group.

Most recently, Preston et al reported the first prospective, multicenter trial for the explicit purpose of determining the pharmacodynamics of an antimicrobial agent [38,39]. Levofloxacin 500 mg o.d. was employed for the treatment of community-acquired lower respiratory tract infections, complicated and uncomplicated skin and soft tissue infections and uncomplicated urinary tract infections. An optimal sampling analysis was performed to guide clinical investigators in the acquisition of serum samples for later analysis. This sample design was employed at all 22 centers, and 1524 serum samples were obtained from 274 patients (of 313 entered into the study). These were analyzed for levofloxacin, and the resultant concentrations were subjected to a population pharmacokinetic analysis. The population means and the associated co-variance matrix were used to estimate the pharmacokinetic parameter values for each patient through the use of maximum a posteriori probability (MAP) Bayesian estimation.

A total of 134 patients had a microbiologically documented infection with an MIC determined for levofloxacin. Logistic regression analysis was performed for clinical and microbiological outcome, employing pharmacodynamic co-variates as well as other demographic and clinical co-variates. The result was clearcut, with the peak/MIC ratio being significantly linked to both clinical and microbiological outcome. As in the Forrest study, there was a high degree of correlation between peak/MIC and AUC/MIC, with a correlation coefficient value exceeding 0.9. Further, a classification and regression tree analysis was performed to see if a breakpoint could be ascertained. It was found that a peak/MIC ratio of >12 was associated with both the best clinical and the best microbiological outcomes (it should also be recognized that the response rate below the ratio of 12 still exceeded 80%). This finding agrees remarkably well with the data of Blaser et al where a peak/MIC ratio of 10 was identified as producing optimal bacteriologic outcomes [28]. The same caveats apply to this study as to that of Forrest et al [36,37]. This was a study of community-acquired infections in moderately ill patients. Its results should not be extrapolated beyond the population studied.

How then, can these two studies be reconciled? The answer comes from the in vitro and animal model studies. An analysis will find AUC/MIC linked to outcome if, on average, the peak/MIC ratio is <10. Otherwise, peak/MIC will appear to be linked to outcome, probably because of suppression of emergence of resistant mutants. In the Forrest study, it is clear that approximately 50% of the patients developed peak/MIC ratios of <10 [36,37]. This is because of the large mixture of patients in this study with *Staphylococcus aureus*, *P. aeruginosa* and *Enterobacter cloacae*, all pathogens with higher MICs for fluoroquinolones, including ciprofloxacin. In the studies by Preston et al, 80% of patients developed peak/MIC ratios of ≥10, almost certainly because of the common pathogens encountered in the community [38,39]. Consequently, it is not surprising that the studies of Forrest et al identified AUC/MIC ratio as the primary pharmacodynamically linked variable for fluoroquinolones, while the studies of Preston et al identified peak/MIC ratio as the dynamically linked variable [36–39].

It should be noted that the guidance for the interpretation of the clinical data came from the in vitro and animal model systems. In part this is because of the clinical reality since it is almost unheard of for different schedules of administration to be tested in clinical trials with adequate numbers of patients. Consequently, in large trials, a drug is administered on a fixed dose and schedule and thus there is a large degree of co-variance between pharmacodynamic variables. In this situation, the peak/MIC ratio cannot be increased without also increasing the AUC/MIC ratio and time>MIC. Therefore, it should not be surprising that different trials can find different dynamically linked variables, depending on the populations and pathogens studied. These differences, it should be noted, are readily explained by the lessons learned from the in vitro and animal model systems.

**CONCLUSIONS**

The pharmacodynamic effect of fluoroquinolones can only be understood in a rational manner if the information regarding them is integrated from at least four sources. Obviously, some measure of potency of the drug for the organisms in question is a critical piece of information, and most clinicians obtain this from a knowledge of the drug’s MIC for clinically important pathogens. More than for most anti-infectives, the pharmacokinetic profile of the fluoroquinolones also plays a major role in determining the observed pharmacodynamics. As has been demonstrated in an animal model of neutropenic infection, it is actually the ratio of these two things (some measure of drug exposure, either peak concentration or AUC, and organism MIC) which mostly determines the observed pharmacodynamic effect [33].
However, it should be realized that the differences in pharmacokinetic profile among the fluoroquinolones are close to the differences in their microbiological activities. For most of the fluoroquinolones, protein binding is low and almost negligible (~30%). However, for some newer drugs, the binding is somewhat greater (~70%) and may adversely impact upon their microbiological activity. This needs to be taken into account in any comparative analysis. Finally, the ability of these drugs to penetrate cells adds many intracellular pathogens to their spectrum of activity. Insight into the physicochemical properties of the drugs which promote this cellular penetration is key for comparing these agents.

Fluoroquinolones are the first new class of antibacterials for which a detailed pharmacodynamic understanding of their action has been gained early in the course of development. This understanding comes from in vitro data, animal models of infection and, uniquely, clinical trial data. This knowledge has led to the optimization of dose schedule for the newer drugs (e.g. demonstrating once-daily dosing is supportable), the rational basis and, perhaps most importantly, the ability to optimize the clinical and microbiological outcome for seriously ill patients on the basis of organism MIC and observed or expected serum drug concentrations.

References