Pharmacodynamic Effects of Subinhibitory Concentrations of Rufloxacin on Bacterial Virulence Factors

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It has been reported that subinhibitory concentrations (sub-MICs) of some fluorquinolones are still capable of affecting the topological characteristics of DNA (inhibition DNA-gyrase) and that this leads to a reduction in some of the factors responsible for bacterial virulence (by means of the disruption of protein synthesis and alterations in phenotype expression), even though the microorganisms themselves are not killed. The present study investigated the ability of sub-MICs of rufloxacin, an orally absorbed monofluorinated quinolone with a long half-life (28 to 30 h), to interfere with the bacterial virulence parameters of adhesiveness, hemagglutination, hydrophobicity, motility, and filamentation, as well as their interactions with host neutrophilic defenses such as phagocytosis, killing, and oxidative bursts. It was observed that Escherichia coli adhesiveness was significantly reduced at rufloxacin concentrations of 1/32 MIC, hemagglutination and hydrophobicity were significantly reduced at concentrations of, respectively, 1/4 MIC and 1/8 MIC, and motility was significantly reduced at concentrations of 1/16 MIC; filamentation was still present at concentrations of 1/4 MIC. Phagocytosis was not affected, but killing significantly increased from 1/2 MIC to 1/8 MIC; oxidative bursts measured by means of chemiluminescence were not affected. The fact that sub-MICs are still effective in interfering with the parameters of bacterial virulence is useful information that needs to be correlated with pharmacokinetic data in order to extend our knowledge of the most effective concentrations that can be used to optimize treatment schedules, for example, single administrations, particularly in noncomplicated lower urinary tract infections.

Bacterial virulence reflects the ability of infecting microorganisms to produce pathological effects in an invaded host. The extent and severity of these effects depend on a number of bacterial cell functions, such as their invasive and adhesive capacities, fimbriation, their outermost surface characteristics and motility, their interaction with host defenses (phagocytosis and killing), the role of metabolism and the release of extracellular and endocellular products, and rates of replication.

In cases of bacterial infection antibiotic therapy is usually adopted, on the assumption that the drug concentrations at the site of infection reach the minimum bactericidal concentration, thus eliminating the virulence of the microorganisms by killing them. In contrast, although the microorganisms do not necessarily die, MICs can inhibit the growth of bacteria and, in most cases, significantly reduce their virulence (49).

With intermittent antibiotic administration, commonly used to treat bacterial infections, pharmacokinetic curves show concentrations that fluctuate on the basis of the dosing schedule. At the site of infection, these concentrations may exceed the in vitro MIC for the invading microorganism for a certain period of time, subsequently decrease more or less rapidly to values corresponding to the MIC, and finally drop to subinhibitory concentrations (sub-MICs), generally between doses. This is particularly true in the case of tissue infections, because tissue concentrations (sub-MICs), generally between doses. This is particularly true in the case of tissue infections, because tissue antibiotic concentrations are frequently lower than those in the blood.

A growing body of evidence (9, 13, 35, 39, 42, 50, 51) strongly suggests that antibiotic concentrations of less than the conventionally determined MICs may still be effective in reducing bacterial virulence by interfering with bacterial cell functions. These findings have generated interest in the effects of exposing bacteria to low concentrations of antibiotics (13, 34), so that pharmacodynamic and pharmacokinetic data can be correlated and therapy can thus be optimized. Rufloxacin is an orally absorbed monofluorinated quinolone with a long half-life (28 to 30 h), consistently high bactericidal concentrations at the site of infection (especially in urine), and good penetration into infected tissues (53, 54). Although some scattered information exists in the literature (5, 7, 17), the effects of sub-MICs of rufloxacin have not yet been fully investigated. The aim of the present study was therefore to investigate the activity of such concentrations on various bacterial cell functions in order to evaluate their ability to reduce bacterial virulence.

MATERIALS AND METHODS

Adhesion assay. Two Escherichia coli strains from clinical isolates (urinary infections) and a reference strain (E. coli ATCC 25922) were used. The MICs were determined in Mueller-Hinton broth by using the tube macrodilution method (38). Each tube contained twofold dilutions of the antibiotic and a final bacterial inoculum of 10⁶ CFU/ml. The tubes were incubated for 18 h at 37°C. The MIC was defined as the lowest concentration of antibiotic that prevented turbidity in the test tube after incubation.

For the adhesion assay, cell suspensions of each organism were prepared from overnight cultures (18 h) in tryptic soy broth (Sigma) at 37°C under static conditions. The organisms were harvested, washed three times in phosphate-buffered saline (PBS), and adjusted to 3 × 10⁶ organisms/ml as determined by direct microscopic counts (interference contrast microscopy) in a Petroff-Hausser bacterial counting chamber. Human periurethral epithelial cells were collected from the sediment of fresh urine (50 ml) of apparently healthy females and resuspended in 20 ml of PBS. The suspensions obtained from three to five subjects were pooled. The epithelial cells were then passed through a needle (0.3 mm diameter) to disrupt cell aggregates and washed three to four times to free them from debris and non-adherent bacteria by using low-speed differential centrifugation (240 × g for 10 min at 21°C).

PBS was added to the washed epithelial cell suspensions to give 10⁴ cells/ml as determined by means of a direct microscopic count (interference contrast microscopy) in a Bürker chamber. The ability of the bacteria to adhere to epithelial

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cells was investigated by mixing together 1:1 volumes of standardized suspensions of bacteria (3 × 10^9/ml) and epithelial cells (3 × 10^7/ml) in polytene tubes. The tubes were rotated end over end at 10 rpm for 60 min at 37°C. The epithelial cells were separated from the nonadherent bacteria by means of differential centrifugation at 100 × g for 5 min. The final bacterial cell pellet was resuspended in a small quantity of PBS, placed on a round microscope coverslip, and dried.

The coverslip with the cells was fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.1) for 60 min at 4°C. After several dehydrations in alcohol, the coverslips underwent critical-point drying, were coated with 200-Å gold, and counted by using a scanning electron microscope (SEM). The adhesiveness of bacterial to epithelial cells was determined (38) by counting the number of epithelial cells with ≥40 adhering bacteria per 100 cells (47). Each test was performed twice. Control epithelial cell suspensions were always included to provide data on the number of bacteria that were already attached (natural acquisition) when the cells were collected.

For the inhibition test, the bacteria were grown in the presence of subinhibitory concentrations of rufloxacin (37°C for 18 h), from 1/2 to 1/128 MIC (1/2, 1/4, 1/8, 1/16, 1/32, 1/64, and 1/128), or with the same amount of medium without any antibiotic (control). The bacteria were harvested by centrifugation at 100 × g and resuspended in PBS at a final concentration of 3 × 10^8 bacteria/ml. They were challenged with epithelial cells as in the adherence assay, and the samples were prepared for SEM analysis.

Hemagglutination assay. Anticoagulated guinea pig and human group A erythrocytes were collected 1:1 in Alsever solution (2.05% glucose, 0.8% sodium citrate, 0.42% NaCl, 0.055% citric acid [pH 6.1]), washed three times in PBS, and finally suspended in saline; the washed erythrocytes were then stored at 4°C and used within 5 days.

The hemagglutination tests were performed by using a slightly modified version of the procedure of Evans et al. (22). Twenty microliters of a 5% (vol/vol) suspension of erythrocytes in saline and 20 μl of the bacterial suspension (10^9 cells/ml) were pipetted onto microscopy slides. The slides were gently rotated at room temperature for 5 min and read after 15 min.

To test for mannose sensitivity, 20 μl of 1% mannose solution was added to a duplicate slide containing unadulterated bacteria. The hemagglutination test was applied to samples of each bacterial strain grown with the different sub-MICs of rufloxacin. The results were recorded as grade 3 when hemagglutination occurred in a very short period of time and was complete (coarse clumping); a reaction with no aggregates or only a few aggregates, i.e., not modifying the overall view of the sample, was regarded as grade 1. When necessary, any residual erythrocytes in the granulocyte preparation were lysed with a 0.15-mol/liter of NH₄Cl solution (pH 7.4). The PMNs were collected by centrifugation at 470 × g and resuspended in PBS at a final concentration of 3 × 10^8 bacteria/ml. They were challenged with epithelial cells as in the adherence assay, and the samples were prepared for SEM analysis.

Phagocytosis and killing. (i) Collection of human PMNs. Peripheral venous blood was drawn from healthy adult donors into heparinized (5 U/ml) syringes. The blood (5 ml) was strained on 3 ml of Polymorphoprep (Nycomed Pharma), and the nonviable leukocytes (PMNs) were separated by density gradient centrifugation.

When necessary, any residual erythrocytes in the granulocyte preparation were lysed with 0.15 mol/liter of NaCl solution (pH 7.4). The PMNs were collected and washed (450 × g for 7 min) and two washes. The pellet was resuspended in 100 μl of PBS (0.02 M phosphate and 0.15 NaCl [pH 7.3]) placed on a round microscope coverslip, and dried. The coverslip was then fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.1) for 60 min at 4°C. After dehydration in graded alcohols, the coverslip underwent critical-point drying, was coated with 200-Å gold, and observed in a SEM.

The microscopic fields to be counted, bacterial size, and the morphology recorded were selected by means of random scanning.

The morphological characteristics before and after incubation with the different sub-MICs of rufloxacin were classified and quantitated, with the observer unaware of the concentration of antibiotic or the duration of incubation. The lengths of the filaments and their proportions in the total number of microorganisms per 100 randomly observed bacteria were recorded; a minimum of eight different fields were examined for each sub-MIC and time. The normal length of E. coli can vary from 1.2 to 2.3 μm; during the mid-cycle division it is about 5 μm.

Organisms of up to 15 μm were classified as short filaments, and those longer than 30 μm as long filaments.

Phagocytosis and bacterial killing. The phagocytic and bacterial killing capacities of the PMNs were determined by using a fluorochrome assay (acridine orange stain), which distinguishes viable and dead microorganisms intracellularly. The procedure of Bellinati-Pires et al. (4) was adopted with slight modifications. Equal volumes of PMNs (2 × 10^6 cells/ml) and preopsonized bacteria (2 × 10^7 bacteria/ml) were mixed in tubes at a ratio of 1:10 (PMN/bacteria) in a final volume of 0.5 ml. The tubes were incubated at 37°C and rotated end over end (6 rpm) for 30 min.

Phagocytosis was stopped by placing each tube in an ice bath and adding 0.5 ml of ice-cold medium to the bacteria-PMN suspension. Noningested bacteria were removed by means of differential centrifugation (100 × g for 7 min) and two washes. The pellet was stained with 200 μl of 14.4-mgliter acridine orange (pH 7.2) (Sigma) in medium for 1 min. Immediately after staining, 1 ml of ice-cold Hanks’ balanced salt solution (HBSS) was added to the PMN suspension, which was then centrifuged at 160 × g for 7 min at 4°C. The cells were washed twice with ice-cold HBSS and kept in an ice bath until microscopic examination.

Acridine orange makes bacteria fluorescent, so phagocytosed bacteria can be easily counted; killed bacteria are also clearly visible because they become yellow-red under UV light, whereas living bacteria are green. After staining with acridine orange to overestimate phagocytosis on the basis of the E. coli CFU attached to the surface of the PMNs but not yet internalized, the techniques of Hed (27) and Goldner et al. (24) were used to quench the extracellular

<table>
<thead>
<tr>
<th>E. coli</th>
<th>Control</th>
<th>1/2</th>
<th>1/4</th>
<th>1/8</th>
<th>1/16</th>
<th>1/32</th>
<th>1/64</th>
<th>1/128</th>
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<tr>
<td>ATCC 25922</td>
<td>38.4</td>
<td>12.1</td>
<td>16.2</td>
<td>19.5</td>
<td>23.3</td>
<td>27.9</td>
<td>36.0</td>
<td>36.2</td>
</tr>
<tr>
<td>Clinical isolate</td>
<td>41.2</td>
<td>20.0</td>
<td>23.7</td>
<td>25.1</td>
<td>30.0</td>
<td>34.0</td>
<td>39.1</td>
<td>44.5</td>
</tr>
<tr>
<td>Clinical isolate</td>
<td>29.4</td>
<td>10.2</td>
<td>14.1</td>
<td>15.0</td>
<td>21.8</td>
<td>26.8</td>
<td>35.0</td>
<td>32.3</td>
</tr>
</tbody>
</table>

Mean ± SD

36.3 ± 6.2 | 14.1 ± 5.2 | 18.0 ± 5.0 | 20.1 ± 4.6 | 25.0 ± 4.3 | 29.5 ± 3.8 | 36.7 ± 2.1 | 37.6 ± 6.2

*a P < 0.05.

*b P < 0.01.

**Table 1. Effects of various subinhibitory concentrations of rufloxacin on E. coli adhesiveness to human epithelial cells**
membrane-adherent microorganism fluorescence by crystal violet (500 µg/ml for 20 min). Since crystal violet does not penetrate PMNs, it does not alter the fluorescence of ingested microorganisms. Just before the observation of each cell sample, a drop of the cell suspension was wet mounted on a microscope slide and sealed with nail varnish. It was then immediately examined under oil immersion with a UV epifluorescence microscope (Leitz) equipped with an excitation filter at 450 to 490 nm, a beam split mirror at 510 nm, and a cut-off filter at 520 nm.

A short time interval lasting no longer than 10 min was established for each slide reading, and if this was not sufficient, another slide was prepared from the ice-cold suspension. A total of 100 PMNs was observed for each slide. The number of cells phagocytizing at least three bacteria/100 PMNs gave the percent of phagocytosis, whereas the average number of bacteria in each phagocytizing cell gave the phagocytic index. The percentage of killed bacteria was obtained by using the following formula: number of dead bacteria/number of dead + live bacteria × 100. The killing index was the average number of dead bacteria per PMN. The same procedure was followed to determine the effect of the exposure of PMNs to different sub-MICs of rufloxacin.

**Measurement of oxidative burst response by chemiluminescence.** Luminol-amplified chemiluminescence (LACL) was investigated by using a slightly modified version of the procedure of Robinson et al. (43) for pathogenic organisms. In brief, 0.1 ml of PMN suspension (10^6 cells/ml) and 0.30 ml of HBSS with Ca^2+ and Mg^2+ plus 0.05 ml 10^{-4} M luminol (diluted from a first stock solution in dimethyl sulfoxide; Sigma) were put into a 3-ml flat-bottomed polystyrene vial. The vial was placed in a lightproof chamber of the Luminometer 1250 (Bio Orlet), and the carousel was rotated to bring the sample in line with the photomultiplier tube in order to record background activity. A suspension of propionated killed Candida albicans cells (2 × 10^7 cells/ml) in a final volume of 0.05 ml was added as a stimulus for oxidative bursts, and the resulting light output in millivolts was continuously recorded on a chart recorder and, simultaneously, by means of a digital printout set for 1- or 10-s recording integrals. All the constituents of the mixture were kept at 37°C during the reaction by means of thermostatically controlled water passing through a polished hollow metal sample holder. No mixing took place during the recordings. The gain control was set to give a reading of 10 mV for a built-in standard. A background subtraction control zeroed the instrument prior to the addition of the opsonized cells. The patterns of LACL responses were determined by calculating the initial means of a digital printout set for 1- or 10-s recording integrals. Thereafter, the area under the curves was integrated. This was done for each sub-MIC and control. Values are expressed as the means of all data ± standard errors of the means.

**Data analysis.** Each test was performed six to nine times for each strain and for each sub-MIC and control. Values are expressed as the means of all data ± standard errors of the means. The statistical significance of the differences was calculated by using the t test and, when necessary, analysis of variance between treatments, followed by multiple pair comparisons according to Dunnett when the differences were statistically significant. Differences were considered statistically significant when the test yielded a value of ≤0.05.

**RESULTS**

During the control test performed before antibiotic challenge, all of the bacterial strains adhered well to human epithelial cells, with a certain degree of variability in the number of bacteria per cell. The mean of the rufloxacin MICs was 1 µg/ml, and after incubation with sub-MICs, bacterial adhesive-ness decreased. The data are summarized in Table 1. To normalize the susceptibilities of the different strains, the data were also expressed as percentages of inhibition versus control, as shown in Fig. 1. As expected, peak inhibition was observed at 1/2 MIC, but inhibition was statistically significant at a MIC as low as 1/32.

The inhibition of hemagglutination and hydrophobicity were significant as low as 1/4 and 1/8 MIC, respectively (Fig. 2). The different radii of the swarming zone read at different times are a measure of the motility of *E. coli* strains. Incubation with 1/2

![FIG. 1. Percent inhibition of *E. coli* adherence to human epithelial cells after incubation with different sub-MICs of rufloxacin.](image)

![FIG. 2. Effects of various rufloxacin sub-MICs of *E. coli* hemagglutination and hydrophobicity (S.A.T., salt aggregation test).](image)

**TABLE 2.** Mean values of swarming zone diameter (mm) at different hours for *E. coli* incubated with different sub-MICs of rufloxacin

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Control</th>
<th>Mean ± SD of swarming zone diameter (mm) for indicated subinhibitory concn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/2</td>
<td>1/4</td>
</tr>
<tr>
<td>0</td>
<td>6.08 ± 0.87</td>
<td>6.00 ± 0.93</td>
</tr>
<tr>
<td>1</td>
<td>6.71 ± 0.87</td>
<td>5.25 ± 0.83</td>
</tr>
<tr>
<td>2</td>
<td>17.48 ± 3.42</td>
<td>6.75 ± 0.66</td>
</tr>
<tr>
<td>3</td>
<td>32.58 ± 8.25</td>
<td>6.88 ± 0.74</td>
</tr>
<tr>
<td>4</td>
<td>55.67 ± 13.20</td>
<td>6.88 ± 0.74</td>
</tr>
<tr>
<td>5</td>
<td>76.58 ± 9.93</td>
<td>6.90 ± 0.81</td>
</tr>
<tr>
<td>6</td>
<td>84.17 ± 2.12</td>
<td>7.17 ± 0.96</td>
</tr>
</tbody>
</table>

* *P* ≤ 0.05.

* *P* ≤ 0.01.
and 1/4 MICs completely inhibited bacterial motility, and this inhibition was significant as low as 1/16 MIC (Table 2).

The morphological changes induced by the sub-MICs of rufloxacin consisted of filamentation. The exposure of *E. coli* to 1/2 MIC and, to a lesser extent, even to 1/4 MIC was capable of inducing filamentation within 2 to 4 h (Table 3). The above parameters relate to the bacteria themselves, but there are also host-bacteria interaction parameters, such as phagocytosis and killing. These were investigated by using fluorescence microscopy and acridine orange fluorochrome. Figure 3A and B show neutrophils with phagocytized *E. coli*. The green bacteria are still alive, whereas those that are orange-red have been killed. Rufloxacin sub-MICs did not change the percentage of phagocytosis or the phagocytic index but significantly increased the killing of bacteria as low as 1/8 MIC (Table 4).

Possible interference with oxidative bursts was investigated by exposing the neutrophils to different sub-MICs of rufloxacin. The findings reported in Table 5 and Fig. 4 show that the rufloxacin sub-MICs did not interfere with this important feature.

**DISCUSSION**

Bacterial virulence is a result of the specific properties of both bacterial and host cells and their reciprocal interactions. Adhesion is considered to be the first step in the sequence of events leading to colonization and subsequent infection (1, 3). The ability to adhere is an important determinant of virulence (21), and the exposure of bacteria to sub-MICs of antibiotics generally weakens this ability (14, 30, 45, 47). This effect has been observed in the case of both quinolones and fluoroquinolones; sub-MICs of oxolinic acid (26), ciprofloxacin (52), pefloxacin (18), enoxacin (6, 49), rufloxacin (7), and lomefloxacin (6) lead to a significant decrease in the adhesion of *E. coli*.

Our rufloxacin findings are similar to those found with other quinolones, but they add further information about the extent of the effect. We observed significantly reduced adhesion at concentrations ranging from 1/2 to 1/32 MIC (corresponding to 0.03 µg/ml), whereas the majority of previous studies considered only 1/2 or 1/4 MIC.

Sub-MICs of antibiotics can exert their antiadhesive effects in different ways. They may inhibit the synthesis or expression of adhesins on the bacterial cell surface, lead to the formation of functionally aberrant adhesins, cause the release of adhesins from the surface of bacterial cells, or modify bacterial shape in a such way as to interfere with the ability of the microorganisms to approach receptors on animal cell surfaces (31, 32).

It has been reported that the fluoroquinolones pefloxacin and enoxacin inhibit adhesion on the basis of the first mechanism of adhesion inhibition (10, 18), so it is likely that rufloxacin also reduces the number of adhesins and thus the possibility of bacterial anchorage (or junction). This is also partially correlated with hemagglutination (an indirect measure of fimbration) and hydrophobicity.
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At 1/2 MIC (and to a lesser extent also at 1/4 MIC), rufloxacin is capable of inducing morphological changes in E. coli, such as different levels of filamentation. Maximum filamentation (=40%) was achieved after 2 h of incubation of bacteria with the 1/2 MIC of rufloxacin (7). Quinolone-induced filamentation has also been observed with the use of ofloxacin (18), enoxacin (25, 49), lomefloxacin (49), ciprofloxacin (16, 25, 52), oxolinic acid (2, 26), and nalidixic acid (26).

The mechanism of filamentation differs from the well-known penicillin-binding protein inhibition induced by ß-lactam antibiotics, probably because initial DNA-gyrase inhibition followed by alterations in DNA topology and synthesis (12, 20, 48, 49) provide the signal necessary for the induction of an SOS response pathway that inhibits cell division (28, 41, 46). In addition to filamentation, the SOS response can involve vacuolation and the leakage of intracellular material (28), which is probably a secondary mechanism of death (19).

The fact that rufloxacin sub-MICs affect some important functions of E. coli is also confirmed by the significant reduction in swarming observed after treatment with 1/2 to 1/16 MIC. Together with adhesiveness, this function is correlated with the pathogenicity of bacteria, because the inhibition of motility also reduces the possibility of the formation of new colonies and the spread of infection from the first point of contact.

It has been shown that the sub-MICS of various antimicrobial agents induce morphological and biochemical changes in bacteria, thus making them more susceptible to phagocytosis and killing (36). There are some data in the literature concerning the effect of quinolone sub-MICS on neutrophil phagocytosis and the killing of gram-positive bacteria (23, 40), but only a few investigations have explored their effects on E. coli. Pre-exposure of E. coli-encapsulated strains to 1/2 MICs of fleroxacin and ciprofloxacin has been found to enhance phagocytosis (6), and sub-MICS of ciprofloxacin can increase the killing of E. coli by neutrophils (37). As for rufloxacin, none of the three strains investigated showed any modification in phagocytosis, but killing was significantly increased in two strains for which the MIC was as low as 1/8 and in one strain for which the MIC was as low as 1/4.

Table 6 summarizes the known data concerning the interactions of quinolone sub-MICS with different virulence parameters. This aspect of nonfluorinated quinolones has not been investigated. The ciprofloxacin, enoxacin, and pefloxacin monofluorinated quinolones have been studied in terms of some parameters, but rufloxacin has been better characterized. The only difluorinated quinolone that has been partially studied is lomefloxacin (11), and trifluorinated quinolones need to be further investigated. Comparison of the data reveals some interesting similarities in effects. Adhesiveness and fimbriation are reduced, and filamentation is increased; phagocytosis remains almost unaffected, but killing is increased; finally, neutrophil oxidative bursts do not vary.

Previous studies have demonstrated that exposure to low concentrations of quinolones may reduce the ability of pathogens to cause clinical symptoms by decreasing the level of production of virulence factors (10, 15). The success of quinolone treatment in patients with sub-MIC levels in their blood (44) supports the clinical significance of our findings. These data must be interpreted in relation to pharmacokinetic behavior when the effectiveness of concentrations reaching mucosal surfaces and other tissues during therapy is considered.

The usual therapeutic dose of rufloxacin is 400 mg, and our MIC for E. coli was 1 µg/ml. The pharmacokinetic curve of a

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Phagocytosis %</th>
<th>Index</th>
<th>Killing %</th>
<th>Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>82.67 ± 4.51</td>
<td>6.27 ± 1.43</td>
<td>37.09 ± 3.76</td>
<td>2.33 ± 0.57</td>
</tr>
<tr>
<td>Rufloxacin MIC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>82.20 ± 7.74</td>
<td>5.46 ± 2.55</td>
<td>52.56 ± 12.15</td>
<td>2.92 ± 1.12</td>
</tr>
<tr>
<td>1/4</td>
<td>83.07 ± 2.81</td>
<td>6.34 ± 1.96</td>
<td>46.24 ± 6.00</td>
<td>2.83 ± 0.80</td>
</tr>
<tr>
<td>1/8</td>
<td>83.73 ± 3.20</td>
<td>6.26 ± 1.48</td>
<td>42.41 ± 5.95</td>
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<tr>
<td>1/16</td>
<td>82.70 ± 4.27</td>
<td>6.31 ± 1.40</td>
<td>38.17 ± 4.90</td>
<td>2.41 ± 0.57</td>
</tr>
<tr>
<td>1/32</td>
<td>80.23 ± 3.32</td>
<td>6.47 ± 1.93</td>
<td>36.15 ± 3.80</td>
<td>2.32 ± 0.66</td>
</tr>
</tbody>
</table>

*P ≤ 0.05.  b P ≤ 0.01.

![LACL curves of PMNs before and after incubation with different sub-MICS of rufloxacin (curves are shown only down to 1/16 MIC to avoid a crowded figure).](http://aac.asm.org/)

**FIG. 4.** LACL curves of PMNs before and after incubation with different sub-MICS of rufloxacin (curves are shown only down to 1/16 MIC to avoid a crowded figure).
single dose of 400 mg (53) indicates that sub-MICs occur after about 48 h (Fig. 5). Given that the average overall effects of sub-MICs of rufloxacin can still be considered significant at levels as low as 1/8 MIC (which corresponds to 0.12 μg/ml), interpolation of this value with the pharmacokinetic curve shows that the effects of sub-MICs may last as long as (approximately) 132 h after drug administration (rufloxacin has a half-life of 28 to 30 h).

Such information can be used to optimize treatment schedules; for example, single administrations for the treatment of noncomplicated lower urinary tract infections such as cystitis.

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