Factors compromising the activity of moxifloxacin against intracellular Staphylococcus aureus

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Objectives: The aim of this study was to determine the intracellular activity of moxifloxacin against a reference strain and a clinical strain and to study the factors compromising the intracellular activity of moxifloxacin.

Methods: The bactericidal activity of moxifloxacin at therapeutic concentrations was studied against extracellular (broth) and intracellular (infected THP-1 monocytes) forms of Staphylococcus aureus and compared with that of levofloxacin. The activity of moxifloxacin was also evaluated in the presence of alkalinizing agents, in intracellular salt medium mimicking the phagolysosomal environment and in cell lysate.

Results: Moxifloxacin, bactericidal against two S. aureus strains (ATCC 25923 and a clinical isolate, Sa2669) in broth, accumulated over 6-fold in monocytes. Against intracellular bacteria, moxifloxacin displayed a markedly reduced activity, not better than levofloxacin, with a maximal reduction of 1 log10 cfu at 5 h. Cellular accumulation of moxifloxacin was not modified by the addition of efflux pump inhibitors or lysosomal alkalinizing agents. Alkalinization of phagolysosomes significantly enhanced intracellular killing by moxifloxacin. The bactericidal activity of moxifloxacin, abolished in the intracellular salt medium, was partially restored when the pH was raised from 5.0 to 7.4. The binding to intracellular components (35%) did not influence the activity of moxifloxacin. In all cases, surviving bacteria remained fully susceptible to the antibiotic.

Conclusions: The defeat of intracellular activity of moxifloxacin against S. aureus appeared to be more substantially related to cellular parameters (acidic pH and composition of the phagolysosomes) than to the intrinsic activity of the drug and to pharmacokinetic properties.

Keywords: fluoroquinolones, cellular pharmacokinetics, intracellular pharmacodynamics, THP-1 monocytes

Introduction

The ability of Staphylococcus aureus to survive within phagocytes can contribute to persistent and recurrent infections.1 Thus, an effective treatment of S. aureus infections requires drugs that are effective against both extra- and intracellular bacteria.2 Fluoroquinolones have a significant activity against intracellular S. aureus.3 However, this activity is much lower than expected on the basis of their extracellular activity and cellular accumulation.3,4 In a previous work, we have demonstrated for levofloxacin that cellular compartmentalization of the drug, phagolysosomal environment and antibiotic binding to cellular components most likely contribute to the failure of intracellular activity.5

Moxifloxacin is currently the most active commercially available fluoroquinolone against Gram-positive organisms. Thus, this drug might be better than levofloxacin for treating staphylococcal infections, particularly those due to quinolone-resistant strains that remain susceptible to moxifloxacin.
Materials and methods

Antibiotics and other chemical agents

Moxifloxacin and levofloxacin were kindly provided by Bayer Pharma (Puteaux, France) and Aventis Pharma (Romainville, France). Verapamil, gemfibrozil, ammonium chloride, monensin and bafilomycin were purchased from Sigma Chemicals (L’Ile d’Abeau, France).

Bacterial strains and measurement of extracellular activity of quinolones

The S. aureus ATCC 25923 reference strain was used to assess the activity of fluoroquinolones. Some experiments were performed with a clinical strain of S. aureus (Sa2669) that was methicillin- and levofloxacin-resistant due to a double-target mutation. Intracellular bacteria surviving after exposure to quinolones were also systematically collected.

MICs and MBCs were determined using the macrodilution method under standard conditions and in Mueller-Hinton (MH) broth adjusted to pH 5.0.5

Time–kill curves were performed up to 24 h in MH broth. In some experiments, killing curves were carried out in cell lysate or in a minimal medium (intracellular salt medium), as described previously.5

Cell infection and assessment of intracellular activity of fluoroquinolones

Experiments were conducted with a THP-1 monocytic cell line (ECACC 88081201, Sigma-Aldrich, Saint Quentin Fallavier, France), according to a procedure described previously.4,5 Infection was achieved by incubating THP-1 monocytes with pre-opsonized bacteria (bacteria/cell ratio = 5/1). Extracellular bacteria were then eliminated by two successive cycles of differential centrifugation. The infected monocytes were next subjected up to 5 h to constant concentrations of fluoroquinolones (from 0.125 to 8 mg/L). In some experiments, ammonium chloride (a lysosomotropic agent, 1 mg/mL) or bafilomycin (an inhibitor of vacuolar ATPase, 250 nM) was added to the medium.

Moxifloxacin uptake by THP-1 cells

Infected THP-1 cells were incubated in RPMI 1640 medium with 4 mg/L moxifloxacin. Cells were separated from the incubation medium by differential centrifugation and cell-associated fluoroquinolone determined in cell lysate by HPLC using fluorescence detection.5 In some experiments, ammonium chloride (1 mg/mL), monensin (a proton ionophore, 20 μM), verapamil (a P-glycoprotein inhibitor, 20 μM) or gemfibrozil (an organic anion transport inhibitor, 0.25 mM) was added to the medium. The uptake of moxifloxacin by THP-1 cells was expressed as the cellular-to-extracellular (C/E) ratio.

Binding of moxifloxacin to cellular components

The extent of moxifloxacin binding to cellular components was determined in cell lysate using the discontinuous ultrafiltration method, as previously described.5

Statistical analysis

A bilateral t-test was performed for comparison between groups and analysis of covariance for comparison between curves. Significance was defined as P < 0.05.

Results

In vitro susceptibility of S. aureus strains to fluoroquinolones

At pH 7.4, the ATCC strain was fully susceptible to levofloxacin and moxifloxacin (MIC = 0.5 and 0.125 mg/L, respectively). The Sa2669 strain was resistant to levofloxacin (MIC = 4 mg/L), but remained susceptible to moxifloxacin (MIC = 1 mg/L). MICs and MBCs of moxifloxacin, measured at pH 5.0, to mimic the pH prevailing in phagolysosomes were 8-fold higher (reference strain) and 16-fold higher (Sa2669 strain) than those at neutral pH. The intracellular-surviving bacteria exhibited the same moxifloxacin susceptibility as the initial inoculum, indicating that they were not resistant mutants, but persisters. The presence of bafilomycin or ammonium chloride did not change the MIC and MBC values of moxifloxacin.

Comparative activity of moxifloxacin and levofloxacin against extra- and intracellular ATCC strain

Against extracellular bacteria, both fluoroquinolones at a concentration of 4 mg/L displayed a rapid (within 1 h) and marked bactericidal effect (reduction of about 3 log₁₀). Intracellular activities of moxifloxacin and levofloxacin were also rapid (Figure 1a). Maximal effect was not significantly different between the two fluoroquinolones and was restricted to a 1 log₁₀ reduction.

Moxifloxacin and levofloxacin killing effects on extracellular (Figure 1b) and intracellular bacteria (Figure 1c) were concentration-dependent. The maximal intracellular activities of levofloxacin and moxifloxacin, achieved after 5 h of incubation for antibiotic levels equal to or higher than 4 × MIC, were quite similar but remained 100-fold lower than the extracellular activities.

Intracellular killing activity of moxifloxacin on Sa2669 strain

Moxifloxacin exerted a reduced activity against the clinical levofloxacin-resistant strain of S. aureus (Sa2669), resulting only in a marginal reduction (0.3 log₁₀) of the initial inoculum, even with a high antibiotic concentration (8 mg/L). For all tested concentrations, the intracellular activity was lower than that measured in MH broth (maximum 1.8 log₁₀).

Cellular accumulation of moxifloxacin

Moxifloxacin penetrated rapidly and accumulated 6-fold within THP-1 cells at equilibrium. Its accumulation was not affected by gemfibrozil, verapamil, ammonium chloride or monensin.
Influence of phagolysosomal pH on the intracellular activity of moxifloxacin

Alkalinizing phagolysosomes with ammonium chloride or bafilomycin (Figure 2a) did not affect the intracellular growth, but consistently enhanced the antibacterial activity of moxifloxacin against intracellular bacteria. The mean reduction of the initial inoculum was significantly higher (2.5 and 3.0 times) after 5 h of incubation with 0.5 mg/L moxifloxacin in the presence of ammonium chloride and bafilomycin, respectively. The same result was observed for higher concentrations of moxifloxacin (2 mg/L) (data not shown).

Antibacterial activity of moxifloxacin in the intracellular salt medium

In the intracellular salt medium (Figure 2b) at pH 5, moxifloxacin (2 mg/L) exerted only a bacteriostatic effect against S. aureus. When the pH of the intracellular salt medium was adjusted to 7.4, the killing effect of moxifloxacin was significantly restored, yielding a mean reduction of \( \log_{10} \) cfu. Nevertheless, this activity remained significantly lower than that observed in MH broth.
Binding to cellular components and influence on the activity of moxifloxacin

Moxifloxacin binding percentage to cellular components was 35% (for two tested concentrations of 0.25 and 0.5 mg/L). The cellular lysate did not affect the bacterial growth and did not modify moxifloxacin activity (Figure 2c).

Discussion

Decreased intracellular activity is a common trait for all fluoroquinolones, as indicated by previous studies and the present one.3,5,7 Our work also demonstrated that under strictly identical conditions, moxifloxacin intracellular activity against S. aureus was not better than levofloxacin, despite a higher intrinsic activity and a higher level of accumulation. Thus, the loss of intracellular activity is quantitatively different between fluoroquinolones. Moreover, moxifloxacin exerted only a bacteriostatic effect against a levofloxacin-resistant and moxifloxacin-susceptible clinical strain (Sa2669). Consequently, accumulation and in vitro susceptibility tests are poor predictors of fluoroquinolone activity against intracellular S. aureus.

In this context, it is important to determine the factors that affect the intracellular activity of fluoroquinolones and to quantify their impact.3 Among them, subcellular compartmentalization might be important. The plateau of intracellular activity (limited to 1 log$_{10}$ reduction) might be related to a saturation of moxifloxacin accumulation into phagolysosomes.9 In our experiments, ammonium chloride, which increases the phagolysosomal pH, monensin, which collapses the transmembrane pH gradient, and gemfibrozil and verapamil, which inhibit efflux pumps, did not markedly influence moxifloxacin accumulation. So, proton trapping and active efflux are probably not involved in intracellular moxifloxacin compartmentalization and saturation of phagolysosomal accumulation appears unlikely.

In contrast, our experimental data tend to demonstrate, in accordance with previous reports,10 the important role of acidic pH in decreasing moxifloxacin activity. Alkalization of S. aureus-containing vacuoles by ammonium chloride or hafloxacin significantly enhanced the intracellular activity of moxifloxacin. Nevertheless, in both cases, the neutralization of the phagolysosomal pH was not sufficient to completely restore the efficacy of moxifloxacin. Other characteristics of the phagolysosomal environment might have a role.

In fact, even if the composition of the intracellular salt medium is probably different from that of the real phagolysosomal medium, our results demonstrated a potential impact on fluoroquinolone activity and strongly suggested that intravacuolar composition and pH acted synergistically to inhibit the antibiotic. Vacular protein content might also modify the activity of moxifloxacin but, experimentally, we failed to find any significant effect of binding on activity. This contrasts with our previous observations for levofloxacin.5

In conclusion, our study confirmed that the intracellular activity of fluoroquinolones is differently impaired according to the molecule. Surprisingly, moxifloxacin was not more efficient than levofloxacin, despite a better antibacterial activity and a higher cellular accumulation. Probably due to different chemical characteristics, moxifloxacin appeared to be more susceptible to the phagolysosomal pH and composition.

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Transparency declarations

None to declare.

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