Influence of a new fluoroquinolone, AF3013 (the active metabolite of prulifloxacin), on macrophage functions against *Klebsiella pneumoniae*: an *in vitro* comparison with pefloxacin

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The efficacy of an antibiotic in the treatment of bacterial infections depends upon the interaction of bacterium, drug and phagocytes. In this study we have investigated the influence of AF3013, a new fluoroquinolone, on the activities of mouse peritoneal macrophages against *Klebsiella pneumoniae*, in comparison with the influence of pefloxacin. Bacterial susceptibility to phagocytosis and intracellular killing were determined after klebsiellae and macrophages had been incubated simultaneously with inhibitory concentrations of both AF3013 and pefloxacin and following pre-exposure of the microorganisms and the macrophages individually to the same concentrations of each drug. Under the experimental conditions used, both AF3013 and pefloxacin potentiated the phagocytic and microbicidal activities of the macrophages, although different mechanisms may be involved.

**Introduction**

Antibiotics used to treat infections, especially in immunocompromised patients, should have a potent antimicrobial effect without impairing host defences such as phagocyte functions. Thus it is preferable that the drugs used should have a stimulatory, rather than an immunosuppressive, effect on the cells of the immune system.

The present study was designed to evaluate the influence of AF3013, a new fluoroquinolone, on the primary functions of macrophages, against *Klebsiella pneumoniae*, comparing it with those of pefloxacin. AF3013 is a thiazetoquinoline carboxylic acid derivative with potent antibacterial activity against a broad spectrum of Gram-positive, Gram-negative and anaerobic bacteria. It is the parent compound of a lipophilic prodrug, prulifloxacin, which, when administered orally, is readily absorbed and hydrolysed to AF3013 *in vivo*.

**Materials and methods**

**Bacteria**

The *K. pneumoniae* strain used in this study was a capsulate, β-lactamase-producing clinical isolate. Bacteria were cultured to mid-exponential phase in brain–heart infusion broth (BHI; Unipath, Milan, Italy), concentrated 10 times in BHI broth containing 30% glycerol, quick-frozen in dry ice–ethanol and stored at –70°C.

**Antibiotics**

AF3013 was kindly supplied by ACRAF/SpA Angelini Ricerche, Rome, Italy. The compound (17.6 mg) was dissolved in 200 mL phosphate buffer (pH 7.3) prepared by mixing 22.3 mL of KH2PO4 9.078 g/L and 77.7 mL of Na2HPO4·2H2O 13.214 g/L. Stock solutions were prepared at 88 mg/L and stored at –70°C (for ≤1 week) until 2 h before use.

Pefloxacin mesylate was kindly provided by Rhône-Poulenc Rorer SpA, Milan, Italy. Aqueous solutions were prepared freshly for each batch of experiments. All solutions were shown to be free of endotoxin by a standard *Limulus* amoebocyte lysate (LAL) assay (BioWhittaker, Walkersville, MD, USA).

**MIC determination**

Antibiotic susceptibility testing was performed by the standardized dilution method in BHI with bacterial inocula...
of $2 \times 10^5$ and $2 \times 10^7$ cfu/mL, as confirmed by colony counts in triplicate. Under these conditions the MIC of AF3013 was 0.03 and 4 mg/L, respectively and that of pefloxacin was 0.5 and 2 mg/L, respectively. In all experiments the bacteria were treated with $1 \times$ MIC of each drug, with bacterial inocula of $2 \times 10^7$ cfu/mL.

**Collection of peritoneal cells**

Unstimulated resident macrophages were harvested from mice by repeated peritoneal lavage with a total of 10 mL of cold Earle’s balanced salt solution supplemented with heparin 100 U/mL. Fluids were pooled and washed three times by centrifugation at 160 g for 10 min. The pellets were then suspended in RPMI 1640 (Gibco Laboratories, Grand Island, NY, USA) supplemented with 10% fetal calf serum (FCS; Gibco), placed in plastic culture dishes (30 mm × 8 mm; Nunc, Copenhagen, Denmark) and incubated for 2 h at 37°C in 5% CO₂ in moist air. Non-adherent cells were removed by vigorous washing. Monolayers were rinsed with Earle’s balanced salt solution and resuspended in fresh RPMI medium. There were approximately $10^6$ macrophages per dish, as assessed by microscopy. Differential cell counts were made both by morphological criteria on smears stained with Diff Quick (Harleco, Gibbstown, NY, USA) and by counting the cells ingesting latex beads after incubation. We used only preparations containing 90% macrophage-like cells as judged by both criteria.

**Labelled bacteria**

Klebsiellae were transferred to 10 mL BHI broth containing 150 μCi of $[^3H]$uracil (specific activity 1165.5 GBq/ mmol; Du Pont de Nemours, NEN Products, Milan, Italy). After 18 h growth at 37°C, radiolabelled microorganisms were centrifuged several times with cold BHI broth and resuspended in fresh RPMI 1640 to a final concentration of $2 \times 10^7$ cfu/mL, as confirmed by colony counts in triplicate.

**Phagocytosis assay**

Radiolabelled klebsiellae ($2 \times 10^7$ cfu/mL) in RPMI 1640 medium with HEPES and 10% fetal calf serum (FCS) containing $1 \times$ MIC of AF3013 or pefloxacin were added to macrophage monolayers ($10^6$ cells/dish) and then incubated on a rocking platform at 37°C in 5% CO₂ in moist air. Drug-free and macrophage-free (total bacteria) controls were set up. After incubation for 30, 60, 90, 120 or 180 min or 24 h, the plates were washed twice with Earle’s buffer to remove free bacteria. Cell cultures were then resuspended in 1 mL of sterile distilled water for 5 min; 100 μL samples of this suspension were placed in a scintillation fluid (Atomlight; Packard, Milan, Italy) and counted by liquid scintillation spectrophotometry. Radioactivity was expressed as cpm/sample. The percentage of phagocytosis at a given sampling time was calculated as:

$$\% \text{ phagocytosis} = \frac{\text{cpm in macrophage pellet}}{\text{cpm in total bacterial pellet}} \times 100$$

**Killing assay**

Mixtures of macrophages and bacterial cells were incubated for 30 min in RPMI 1640 medium with HEPES and 10% FCS, for phagocytosis to take place. The macrophage monolayers were washed vigorously several times with Earle’s buffer to remove free extracellular bacteria. A sample of the cells containing bacteria was lysed by adding sterile water and a viable count of intracellular klebsiellae was performed ($t_0$). The cells were then incubated further in RPMI 1640 medium containing $1 \times$ MIC of AF3013 or pefloxacin and, at intervals ($t_x$), viable counts of surviving intracellular bacteria were measured in the same way. Drug-free controls were set up. The macrophage killing values were expressed as the survival index (SI), calculated from the formula:

$$\text{SI} = \frac{\text{no. of cfu at } t_0 + \text{ no. of cfu at } t_x}{\text{no. of cfu at } t_0}$$

According to this formula, if 100% killing took place, the SI would be 1.

**Effect on macrophage functions of pre-exposure of macrophages and bacteria to AF3013 or pefloxacin**

To differentiate between any effect of the antibiotics on the bacteria and the macrophages, the experiments were conducted after exposure of each of them to AF3013 or pefloxacin before they were incubated together. The bacteria were inoculated into 10 mL of BHI broth (controls) or BHI containing $1 \times$ MIC of the drug. After 1 h at 37°C, the bacteria were centrifuged at 2000g for 15 min, washed with Earle’s buffer to remove the drug and adjusted to $2 \times 10^7$ cfu/mL. In another set of experiments, the macrophage monolayers were pre-incubated at 37°C in 5% CO₂ in moist air for 1 h with 1 mL of AF3013 or pefloxacin ($1 \times$ MIC) or 1 mL of sterile balanced salt solution as control. After 1 h the monolayers were washed several times with Earle’s buffer to remove the antibiotic. Pre-exposed bacteria were added to macrophages, and bacteria were added to pre-exposed macrophages, then the plates were incubated at 37°C on a rocking platform for 30 min to 24 h. A control system was assayed in parallel. The phagocytic and bactericidal activities of phagocytes were determined as described above.
AF3013 vs pefloxacin effect on macrophage activity

**Statistical analysis**

Each test was carried out in quadruplicate and compared with control systems that contained no AF3013 or pefloxacin. Results are expressed as the mean ± S.E.M. for 10 separate experiments. Statistical evaluation of the differences between test and control results was performed by an analysis of variance by Tukey’s test.

**Results**

The presence of 1 × MIC (4 mg/L) of AF3013 significantly enhanced the phagocytic activity of macrophages when the antibiotic and the klebsiellae were added simultaneously to phagocytes (Table I, column A). The proportion of engulfed bacteria increased significantly (P < 0.01) after 60 min and up to 180 min, compared with antibiotic-free controls. Table I (column B) shows that pre-exposure of phagocytes to AF3013 for 60 min led to a significantly greater phagocytosis of the klebsiellae for the entire period of observation in comparison with the control system (P < 0.01). Similarly, when the klebsiellae were pre-exposed to AF3013, there was a significant enhancement of phagocytosis from 60 min to 24 h (Table I, column C; P < 0.01).

1 × MIC of AF3013 enhanced the intracellular killing of *K. pneumoniae* by macrophages under the experimental conditions used (Table II). In the drug-free system, the phagocytosed microorganisms remained viable after incubation for >30 min, with SIs consistently being >2, indicating intracellular survival. The presence of the drug produced a significantly greater bactericidal effect than the controls, achieving mean killing values of 97−98% (Table II, column A; P < 0.01).

When phagocytes were pretreated with AF3013 for 1 h before their exposure to bacteria, the survival rate was

### Table I. Effect of AF3013 on macrophage phagocytosis of *K. pneumoniae*; all data are means ± S.E.M.

<table>
<thead>
<tr>
<th>Time of exposure</th>
<th>Controls</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 min</td>
<td>5.22 ± 0.97</td>
<td>5.73 ± 1.87</td>
<td>7.95 ± 0.4 b</td>
<td>6.5 ± 0.05</td>
</tr>
<tr>
<td>60 min</td>
<td>4.28 ± 1.61</td>
<td>7.03 ± 2.3 a</td>
<td>14.6 ± 0.35 a</td>
<td>8.8 ± 0.31 a</td>
</tr>
<tr>
<td>90 min</td>
<td>3.5 ± 0.71</td>
<td>6.46 ± 2.4 a</td>
<td>12.12 ± 3.1 a</td>
<td>13.1 ± 0.79 a</td>
</tr>
<tr>
<td>2 h</td>
<td>2.68 ± 0.45</td>
<td>5 ± 0.98 a</td>
<td>10.6 ± 2.6 a</td>
<td>13.4 ± 1.4 a</td>
</tr>
<tr>
<td>3 h</td>
<td>2.65 ± 0.93</td>
<td>4.45 ± 1.06 a</td>
<td>5.8 ± 0.38 a</td>
<td>11.7 ± 0.65 a</td>
</tr>
<tr>
<td>24 h</td>
<td>2 ± 0.02</td>
<td>1.56 ± 0.02</td>
<td>4 ± 0.09 a</td>
<td>8.3 ± 0.8 a</td>
</tr>
</tbody>
</table>

aSignificantly different from the controls (P < 0.01).

bSignificantly different from the controls (P < 0.05).

A, Addition of labelled klebsiellae and 4 mg/L of AF3013 (1 × MIC) to macrophage monolayers; B, addition of labelled klebsiellae to macrophage monolayers following 1 h pre-exposure of phagocytes to AF3013 4 mg/L (1 × MIC); C, addition of labelled klebsiellae to macrophage monolayers following 1 h pre-exposure of *K. pneumoniae* to AF3013 4 mg/L (1 × MIC).

### Table II. Effect of AF3013 on macrophage killing of intracellular klebsiellae expressed as survival index and as a percentage; all data are means ± S.E.M.

<table>
<thead>
<tr>
<th>Time of exposure</th>
<th>Controls</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 min</td>
<td>1.68 ± 0.2 (32%)</td>
<td>1.04 ± 0.04 (96%) a</td>
<td>1.37 ± 0.07 (63%) a</td>
</tr>
<tr>
<td>60 min</td>
<td>&gt;2</td>
<td>1.03 ± 0.01 (97%) a</td>
<td>1.54 ± 0.14 (46%) a</td>
</tr>
<tr>
<td>90 min</td>
<td>&gt;2</td>
<td>1.02 ± 0.03 (98%) a</td>
<td>1.76 ± 0.13 (23%) a</td>
</tr>
<tr>
<td>2 h</td>
<td>&gt;2</td>
<td>1.02 ± 0.06 (98%) a</td>
<td>&gt;2</td>
</tr>
<tr>
<td>3 h</td>
<td>&gt;2</td>
<td>1.03 ± 0.05 (97%) a</td>
<td>&gt;2</td>
</tr>
<tr>
<td>24 h</td>
<td>&gt;2</td>
<td>1.02 ± 0.02 (98%) a</td>
<td>&gt;2</td>
</tr>
</tbody>
</table>

aSignificantly different from the controls (P < 0.01).

A, addition of klebsiellae and AF3013 4 mg/L (1 × MIC) to macrophage monolayers; B, addition of klebsiellae to macrophage monolayers following 1 h pre-exposure of macrophages to AF3013 4 mg/L (1 × MIC).
significantly lower ($P < 0.01$) than that of the controls, until 90 min (Table II, column B). Afterwards, the SIs for the controls and the drug systems were similar (SI > 2), indicating the absence of killing.

Pretreatment of *K. pneumoniae* for 1 h with AF3013 markedly increased the microbicidal activity of macrophages throughout the entire 180 min period of observation (Figure). As expected, when klebsiellae were incubated in the absence of macrophages and antibiotic, there was substantial bacterial growth. In the plates containing both macrophages and klebsiellae, but not the fluoroquinolone, there was a slight decrease in the number of viable organisms during the first 1 h of incubation, followed by a marked increase in the number of viable intraphagocytic klebsiellae within 3 h. When bacteria were pre-exposed for 1 h to AF3013, in the absence of macrophages, the number of viable klebsiellae decreased markedly only during pretreatment; afterwards there was no bacterial regrowth up to 3 h. AF3013 was clearly bactericidal against phagocytosed pre-exposed klebsiellae: the number of bacteria within the macrophages decreased further during the first 30 min of incubation. Throughout the entire 3 h period of incubation, bacterial viability was significantly lower ($P < 0.01$) than that with antibiotics alone (Figure).

The effects of $1 \times MIC$ (2 mg/L) of pefloxacin on phagocytosis of *K. pneumoniae* by macrophages under the three experimental conditions used are shown in Table III. When pefloxacin and klebsiellae were added simultaneously to the cell monolayers, phagocytosis was significantly enhanced between 1 h and 3 h of incubation (Table III, column A; $P < 0.01$). Pre-exposure of cell monolayers to pefloxacin for 60 min did not affect the phagocytic capacity of macrophages (Table III, column B). In contrast, pretreatment of klebsiellae with pefloxacin for 1 h increased their phagocytic uptake by macrophages at all times from 30 min to 24 h (Table III, column C).

The SIs of controls and pefloxacin cultures after various periods of exposure are shown in Table IV. When pefloxacin was added to macrophages after phagocytosis of klebsiellae (Table IV, column A), bactericidal activity (90–97% killing) was significantly greater than that observed in the drug-free controls throughout the period of observation ($P < 0.01$). Pefloxacin pretreatment of macrophages (Table IV, column B) also led to a significant decrease in SIs compared with the controls for up to 2 h ($P < 0.01$); after this the killing of intracellular klebsiellae was no greater than that in the controls. When klebsiellae were pretreated for 1 h with pefloxacin a pattern similar to that observed for AF3013 was detected (Figure).

**Discussion**

The effects of quinolones on phagocyte antimicrobial functions have been extensively studied. Some quinolones, including norfloxacin, ciprofloxacin, ofloxacin, levofloxacin and trovafloxacin, are effective against facultative intracellular pathogens, such as mycobacteria and *Legionella spp.*, and against *Staphylococcus aureus* etc., which, in certain circumstances, can survive within phagocytic cells. 7-10 We have shown previously that both AF3013 and pefloxacin can enter phagocytic cells rapidly by a passive process, achieving concentrations in the cell greater than

**Figure.** Influence of bacterial exposure to AF3013 or pefloxacin on the viability of *Klebsiella pneumoniae* in the presence or absence of mouse peritoneal macrophages. ▲, Control (no macrophages and no antibiotic); ○, macrophages only; ●, pefloxacin only; ■, AF3013 only; ♦, macrophages and pefloxacin; □, macrophages and AF3013. At 30, 60, 90, 120 and 180 min there were significant differences ($P < 0.01$) between macrophages alone (○) and macrophages plus antibiotics (●, ■).
AF3013 vs pefloxacin effect on macrophage activity

**Table III.** Effect of pefloxacin on macrophage phagocytosis of *K. pneumoniae*; all data are means ± S.E.M.

<table>
<thead>
<tr>
<th>Time of exposure</th>
<th>Controls</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 min</td>
<td>5.22 ± 0.97</td>
<td>6.1 ± 0.11</td>
<td>6 ± 0.1</td>
<td>7.78 ± 0.41&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>60 min</td>
<td>4.28 ± 1.61</td>
<td>9.3 ± 0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.9 ± 0.2</td>
<td>13.18 ± 0.96&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>90 min</td>
<td>3.5 ± 0.71</td>
<td>8.75 ± 2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.81 ± 0.15</td>
<td>14.31 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 h</td>
<td>2.68 ± 0.45</td>
<td>8.04 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.87 ± 0.18</td>
<td>13.8 ± 0.99&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3 h</td>
<td>2.65 ± 0.93</td>
<td>7.10 ± 0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.76 ± 0.2</td>
<td>10.56 ± 0.89&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>24 h</td>
<td>2 ± 0.02</td>
<td>1.95 ± 0.07</td>
<td>1.98 ± 0.03</td>
<td>6.42 ± 0.55&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Significantly different from the controls (*P* < 0.01).
<sup>b</sup>Significantly different from the controls (*P* < 0.05).

A, Addition of labelled klebsiellae and pefloxacin 2 mg/L (1 × MIC) to macrophage monolayers; B, addition of labelled klebsiellae to macrophage monolayers following 1 h pre-exposure of phagocytes to pefloxacin 2 mg/L (1 × MIC); C, addition of labelled klebsiellae to macrophage monolayers following 1 h pre-exposure of *K. pneumoniae* to pefloxacin 2 mg/L (1 × MIC).

**Table IV.** Effect of pefloxacin on macrophage killing of intracellular klebsiellae expressed as survival index and as a percentage; all data are means ± S.E.M.

<table>
<thead>
<tr>
<th>Time of exposure</th>
<th>Controls</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 min</td>
<td>1.68 ± 0.2 (32%)</td>
<td>1.06 ± 0.07 (94%)&lt;sup&gt;y&lt;/sup&gt;</td>
<td>1.37 ± 0.09 (63%)&lt;sup&gt;y&lt;/sup&gt;</td>
</tr>
<tr>
<td>60 min</td>
<td>&gt;2</td>
<td>1.04 ± 0.01 (96%)&lt;sup&gt;y&lt;/sup&gt;</td>
<td>1.7 ± 0.11 (30%)&lt;sup&gt;y&lt;/sup&gt;</td>
</tr>
<tr>
<td>90 min</td>
<td>&gt;2</td>
<td>1.03 ± 0.03 (97%)&lt;sup&gt;y&lt;/sup&gt;</td>
<td>1.7 ± 0.08 (30%)&lt;sup&gt;y&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 h</td>
<td>&gt;2</td>
<td>1.03 ± 0.06 (97%)&lt;sup&gt;y&lt;/sup&gt;</td>
<td>1.85 ± 0.07 (15%)&lt;sup&gt;y&lt;/sup&gt;</td>
</tr>
<tr>
<td>3 h</td>
<td>&gt;2</td>
<td>1.05 ± 0.05 (95%)&lt;sup&gt;y&lt;/sup&gt;</td>
<td>&gt;2</td>
</tr>
<tr>
<td>24 h</td>
<td>&gt;2</td>
<td>1.10 ± 0.02 (90%)&lt;sup&gt;y&lt;/sup&gt;</td>
<td>&gt;2</td>
</tr>
</tbody>
</table>

<sup>y</sup>Significantly different from the controls (*P* < 0.01).

A, Addition of klebsiellae and pefloxacin 2 mg/L (1 × MIC) to macrophage monolayers; B, addition of klebsiellae to macrophage monolayers following 1 h pre-exposure of macrophages to pefloxacin 2 mg/L (1 × MIC).

However, high intracellular drug concentrations do not necessarily translate into potent anti-infective activity. After having penetrated into phagocytes, an antibiotic must remain stable and capable of expressing its microbiidal activity against ingested bacteria. The present study was undertaken to compare the effects of AF3013, a new fluoroquinolone with a potent and broad antibacterial activity<sup>2,3</sup> on phagocytosis and microbiidal activity of mouse peritoneal macrophages, with that of pefloxacin.

When AF3013 or pefloxacin, at inhibitory concentrations, was incubated with *K. pneumoniae* and macrophages, bacterial phagocytosis by the macrophages was enhanced significantly. The mechanisms responsible for this enhancement are not known, but might relate to the way in which pefloxacin enters Gram-negative bacteria and binds to their outer membrane.<sup>13</sup> These observations are in accordance with the data of Desnottes et al.,<sup>14</sup> who showed that low concentrations of pefloxacin affect some surface properties of Gram-negative bacteria, such as hydrophobicity, negative charge, cell wall and adhesins, and facilitate bacterial uptake by cells.

The two fluoroquinolones had similar bactericidal activity against phagocytosed klebsiellae (Tables II–IV, columns A). In the controls the number of viable intracellular bacteria increased progressively (SIs were consistently >2 after 30 min of incubation), whereas the number of intracellular organisms that survived after incubation in the presence of either antibiotic decreased significantly throughout the period of observation: both quinolones killed >96–97% of the phagocytosed klebsiellae (*P* < 0.01). These results compare favourably with those of previous reports describing enhanced intracellular killing by NM394 (AF3013), pefloxacin and other fluoroquinolones of Gram-
negative pathogens such as *Pseudomonas aeruginosa*, 15–17 *K. pneumoniae*, 8,11 and *Legionella pneumophila*. 9 In contrast, others have reported low or no intracellular activity of the quinolones. 18 The enhancement of the antimicrobial intracellular activity detected in this study confirms the capacity of both drugs to penetrate and remain within phagocytic cells. 11,12,15 Their elution from macrophages occurred relatively slowly, 40% of the drug still being present in cells 60 min after removal of the extracellular drug. 12 Therefore, once either quinolone was concentrated intracellularly, it could kill the bacteria directly or make them more susceptible to the bactericidal effect of the phagocytes.

In order to differentiate between the effects of the quinolones on *K. pneumoniae* and those on the macrophages, the phagocytic and bactericidal activities of the macrophages were assessed following pre-exposure of the macrophages and klebsiellae individually to either AF3013 or pefloxacin. The significant increase in phagocytosis of klebsiellae following pre-exposure of phagocytes to AF3013 (Table I, column B) compared with either the control or the pefloxacin systems suggests that AF3013 might act directly on macrophages, possibly by binding the cellular membrane and thereby increasing the phagocytic capacity. Further investigations will be required to confirm this hypothesis. Pretreatment of macrophages with AF3013 or pefloxacin resulted in significantly enhanced killing of intracellular klebsiellae. An indirect effect on macrophage activity via changes in bacteria can be ruled out since, under these experimental conditions, there was no direct contact between klebsiellae and antibiotic outside the phagocytes. Consequently, these data suggest that, once inside the cells, the antibiotics can act on intracellular bacteria, rendering them more susceptible to lytic macrophage enzymes. In normal phagocytes, the rate of intracellular killing depends on the number of bacteria ingested; the higher SI values observed within 2 h for the pefloxacin-pretreated macrophages (Table IV, column B) must, therefore, be considered in relative terms, because under these experimental conditions, the number of ingested bacteria was significantly lower (Table III, column B) than that in AF3013-pretreated macrophages (Table I, column B).

Pre-exposure of *K. pneumoniae* to AF3013 or pefloxacin for 1 h induced a marked increase in bacterial phagocytosis (Tables I–III, columns C): the pretreated bacteria were more susceptible to phagocytosis than the untreated ones, indicating that the quinolones had a direct effect on the klebsiellae. Light microscopy revealed that *K. pneumoniae* exposed for 1 h to 1 × MIC of either of the fluoroquinolones had some morphological abnormalities such as short filaments or forms increased in size (data not shown). This quinolone-induced phenotypic and biochemical damage in *K. pneumoniae* might facilitate phagocytosis. In addition, pretreated *K. pneumoniae*, once phagocytosed, were more efficiently killed (Figure): the combined microbicidal activity of a quinolone and macrophages resulted in a further significant decrease (*P* < 0.01) in intracellular viable klebsiellae in comparison with the bacteria–macrophage antibiotic-free systems (Figure).

In conclusion, both quinolones at inhibitory concentrations modified the bacteria–phagocyte interaction, enhancing the efficiency of the macrophages, although possibly by different mechanisms. In particular, AF3013 acts directly on either the bacterial cell or the phagocytes. It will be interesting to see whether this enhanced efficiency is mirrored in clinical results.

References


AF3013 vs pefloxacin effect on macrophage activity


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