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Contrasting effects of human THP-1 cell differentiation on levofloxacin and moxifloxacin intracellular accumulation and activity against *Staphylococcus aureus* and *Listeria monocytogenes*

Sébastien Van de Velde[1]†, Hoang Anh Nguyen[1,2]†, Françoise Van Bambeke[1]*, Paul M. Tulkens[1], Jean Grellet[2], Véronique Dubois[2], Claudine Quentin[2] and Marie-Claude Saux[2]

[1]Unité de pharmacologie cellulaire et moléculaire, Université catholique de Louvain, Bruxelles, Belgium; [2]Laboratoire de Pharmacocinétique et de Pharmacie Clinique et Laboratoire de Microbiologie, Université Victor Ségalen Bordeaux 2, Bordeaux, France

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***Background and aims*: *Listeria monocytogenes* and *Staphylococcus aureus* invade and multiply in THP-1 monocytes. Fluoroquinolones accumulate in these cells, but are less active against intracellular than extracellular forms of *L. monocytogenes* and *S. aureus*. We examined whether differentiation of THP-1 monocytes into adherent, macrophage-like cells increases fluoroquinolone uptake and activity.**

***Methods*: THP-1 monocytes were differentiated with phorbol myristate acetate (PMA) and compared with unstimulated cells for: (i) moxifloxacin and levofloxacin accumulation; and (ii) activity against phagocytosed *L. monocytogenes* and *S. aureus* (5 h contact).**

***Results*: The differentiation of THP-1 monocytes caused: (i) a 3- to 4-fold increase in moxifloxacin uptake and a significant increase in its activity against intracellular *L. monocytogenes* (from 1.3 $\log_{10}$ to 2.1 $\log_{10}$ cfu decrease compared with the post-phagocytosis inoculum), but not against *S. aureus* (1.0–1.2 $\log_{10}$ cfu decrease throughout); and (ii) no change in levofloxacin accumulation and intracellular activity against either *L. monocytogenes* or *S. aureus*.**

***Conclusions*: Although differentiation of monocytes enhances the uptake and activity of moxifloxacin against *L. monocytogenes*, this cannot be extended to other intracellular bacteria and to levofloxacin. These results further demonstrate that antibiotic intracellular accumulation and activity are not necessarily linked and suggest that intracellular drug and pathogen combinations must be studied individually.**

Keywords: phorbol myristate acetate, macrophages, phagocytosis, cytosol, phagolysosomes

Introduction

Listeria monocytogenes and *Staphylococcus aureus* are known to survive and even multiply inside phagocytic cells, which is considered a major cause for chronic, relapsing and/or invasive infections.[1] *In vitro* studies show that the intracellular forms of these bacteria are considerably less susceptible to antibiotics than the extracellular ones, even for drugs that accumulate in cells, such as fluoroquinolones.[2] The differentiation of human THP-1 monocytes into adherent, macrophage-like cells by interferon-γ increases the activity of sparfloxacin against phagocytosed *L. monocytogenes*.[3] We wondered whether this effect (i) would also be observed after differentiation by a less-specific activator, such as phorbol ester,[4] and (ii) could be extended to other fluoroquinolones and to other intracellular bacteria, such as *S. aureus*. *L. monocytogenes* and *S. aureus* gain access to and thrive in distinct subcellular compartments, namely the cytosol and phagolysosomes, respectively.[2,5] Fluoroquinolones accumulated by macrophages are found in the cytosol after cell fractionation but act readily not only against intracellular *L. monocytogenes* but also, and to a similar extent, against intracellular *S. aureus* in unstimulated cells, which

*Correspondence address. UCL 7370 Avenue E. Mounier 73, B-1200 Bruxelles, Belgium. Tel: +32-2-764-7378; Fax: +32-2-764-7373;
E-mail: vanbambeke@facm.ucl.ac.be
†These authors contributed equally to this work.

suggests that they can access the phagolysosomal compartment in infected cells.[2,5] The present study was therefore initiated to compare unstimulated and phorbol-ester-differentiated THP-1 cells for the uptake of two representative fluoroquinolones, levofloxacin and moxifloxacin, and for their activity against phagocytosed *L. monocytogenes* and *S. aureus*.

Materials and methods

Source of products

Levofloxacin and moxifloxacin were procured as microbiological standards from Sanofi-Aventis, Paris, France and BayerHealthCare GmbH, Leverkusen, Germany. Culture media and sera were from Invitrogen Inc., Carlsbad, CA, USA and, unless specified otherwise, other products were from Sigma-Aldrich, St Louis, MO, USA, or E. Merck AG, Darmstadt, Germany.

Cells and differentiation method

THP-1 monocytes were maintained as described previously.[3] Differentiation and adherence were obtained by incubation with phorbol 12-myristate 13-acetate (PMA) (100–160 nM, Sigma-Aldrich) for 1–3 days at 37°C.[6] Differentiation was followed by morphological observation of the cells and by following the expression of the leucocyte integrin CD11b (macrophage-1 antigen) by semi-quantitative immunofluorescence [monoclonal FITC-labelled anti-CD11b antibody, Coultronics, Margency, France (presently Beckman Coulter, Inc., Fullerton, CA, USA)].

Bacteria and determination of MICs

L. monocytogenes (haemolysin-producing strain EGD serotype 1/2a) and *S. aureus* (ATCC 25923) were obtained and used as described previously.[2] MICs were determined at pH 7.4 (*L. monocytogenes* and *S. aureus*) and at pH 5.0 (*S. aureus*) by microdilution.[2]

Uptake of quinolones

Uninfected cells were exposed to quinolones at a final concentration of 4 mg/L in complete culture medium. Cells were collected and samples were prepared as described previously[3] for unstimulated, non-adherent THP-1 cells and as described for murine J774 macrophages[2] for differentiated, adherent THP-1 cells. Fluoroquinolones were assayed by HPLC and fluorescence detection (λ_{ex} = 292 and 296 nm and λ_{em} = 496 and 504 nm for levofloxacin and moxifloxacin, respectively). Cell drug contents were expressed by reference to the total cell protein content [bicinchoninic acid method (BCA protein kit assay, Pierce, Rockford, IL, USA)].

Infection and measurement of intracellular activities of quinolones

Experiments were conducted as described previously,[2] except that extracellular *S. aureus* were removed by washing once with 2 mL of warm PBS, followed by a 20 min incubation with lysostaphin (5 mg/L). For unstimulated, non-adherent THP-1 cells, washes were done by centrifugation (1400 rpm and 5 min) in PBS, whereas medium draining followed by gentle cell-sheet washing with PBS was used for differentiated, adherent THP-1 cells (as described previously for murine J774 macrophages).[2] Results were expressed as the variation in the number of cfu collected per mg of protein of cells after 5 h of incubation, in comparison with the value found immediately after phagocytosis and removal of extracellular bacteria.

Results

PMA-induced differentiation of THP-1 cells

After incubation of THP-1 monocytes with PMA, >90% of the cells became adherent to culture dishes. Cellular proliferation markedly decreased, and morphological changes consistent with differentiation were observed (macrophage-like appearance with a diffused and enlarged shape and occurrence of a large number of intracellular vacuoles).[4] Differentiation was confirmed by observing the expression of CD11b (data not shown).

Cellular uptake of quinolones

Figure 1 shows the time profiles of the uptake of moxifloxacin and levofloxacin by unstimulated and differentiated cells over a 5 h period. The accumulation was maximal after only 30 min of incubation and remained almost constant thereafter. There was a 3- to 4-fold increase in the accumulation level of moxifloxacin in differentiated cells, compared with unstimulated ones. This effect was not seen for levofloxacin, the cellular content of which was ~60% lower than that of moxifloxacin in unstimulated cells.

Susceptibility of L. monocytogenes and S. aureus to fluoroquinolones in broth

The MICs of moxifloxacin and levofloxacin for *L. monocytogenes* (measured at pH 7.4) were 0.5 and 2.0 mg/L, respectively.

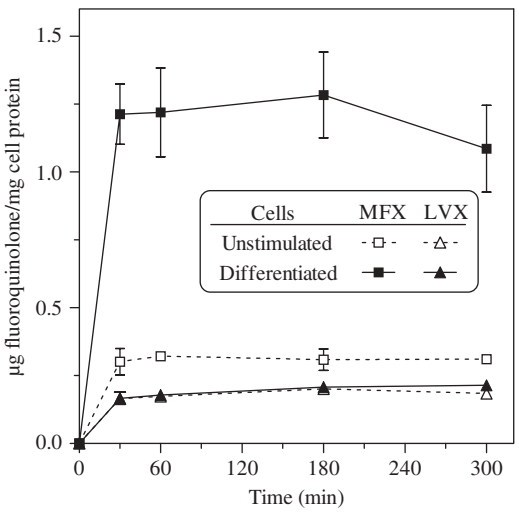

Figure 1. Cellular content of fluoroquinolones in unstimulated (open symbols and broken lines) and PMA-differentiated (closed symbols and continuous lines) THP-1 cells exposed to moxifloxacin (MXF; squares) or levofloxacin (LVX; triangles) at a fixed extracellular concentration (4 mg/L) for the time periods indicated. Each value is the mean of three independent experiments (± SEM; n = 3). Based on measurements of the cell volume corresponding to 1 mg of cell protein {4.9 µL/mg protein and 6.1 µL/mg protein for unstimulated [1 pL[10] and 206 pg protein (this study) per cell] and differentiated cells [2.8 pL[10] and 466 pg protein (this study) per cell]}, the accumulation factors at 300 min are 15.8 ± 1.9 and 44.9 ± 11.4 for moxifloxacin and 9.4 ± 1.6 and 8.8 ± 1.3 for levofloxacin in unstimulated and differentiated cells, respectively.

For *S. aureus*, MICs were 0.125 and 0.5 mg/L for moxifloxacin and levofloxacin at pH 7.4, respectively, and 2 mg/L for both antibiotics at pH 5.0. The MBCs of moxifloxacin and levofloxacin for *S. aureus* were 1 $\log_2$ dilution higher than their MICs in all cases.

Phagocytosis, intracellular growth and susceptibility of phagocytosed L. monocytogenes and S. aureus to fluoroquinolones

Unstimulated and differentiated THP-1 cells phagocytosed *L. monocytogenes* and *S. aureus* to a similar extent (~10^6 cfu/mg of cell protein). The fluoroquinolone intracellular activity was measured over the same time frame as their accumulation (5 h). *L. monocytogenes* post-phagocytosis growth in the absence of antibiotics was similar between both cell types (~1 $\log_{10}$ cfu increase; Figure 2). In contrast, the growth of *S. aureus* was almost completely impaired in differentiated versus unstimulated cells. In unstimulated cells, the addition of moxifloxacin or levofloxacin (4 mg/L) allowed for a significant but similar reduction in the bacterial count. In differentiated cells, moxifloxacin was significantly more active than levofloxacin towards *L. monocytogenes*, but this was not the case for *S. aureus* (killing of 1 $\log_{10}$ cfu in all cases). Levofloxacin activity was not modified by differentiation.

As the lack of effect exerted by differentiation on the activity of fluoroquinolones towards *S. aureus* could have resulted from the use of a single extracellular concentration far above the MIC, experiments were repeated using increasing extracellular concentrations of both moxifloxacin and levofloxacin [from 1 to 16 times their MIC (as measured at pH 7.4 in broth)]. There was a concentration-dependent decrease in intracellular cfu up to an extracellular concentration of ~8-fold the MIC, at which point a plateau (~1.2 $\log_{10}$ cfu decrease from the post-phagocytosis inoculum) was reached. No difference between levofloxacin and moxifloxacin was noted when data were analysed after normalization for differences in MICs, and no difference was noted either between unstimulated or differentiated THP-1 cells. The persistence of a large bacterial intracellular load was not the result of the selection of a subpopulation with decreased susceptibility because bacteria collected from cells after 5 h of incubation at the maximal concentration of either moxifloxacin or levofloxacin had unaltered MICs compared with the original inocula.

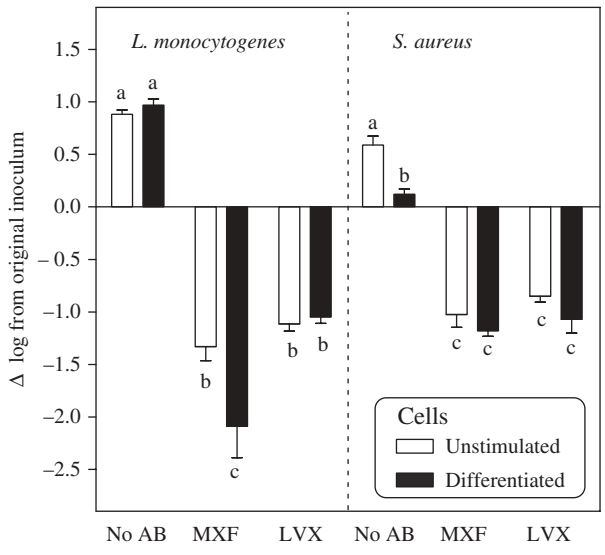

Figure 2. Growth or killing of *L. monocytogenes* (left) or *S. aureus* (right) phagocytosed by control (white bars) or PMA-activated (black bars) macrophages maintained for 5 h in the absence of antibiotic (no AB) or in the presence of moxifloxacin (MFX) or levofloxacin (LVX) at a fixed concentration (4 mg/L). The ordinate shows the changes in cfu from the original, post-phagocytosis inoculum. Values are given as arithmetic means ± SEM. Statistical analysis: bars with different letters are significantly different from each other ($P < 0.05$) by one-way analysis of variance (ANOVA with Tukey's *post hoc* test for multiple comparisons; data for *L. monocytogenes* and *S. aureus* are analysed separately).

Discussion

Mononuclear phagocytes (monocytes and macrophages) are engaged in host defence against infectious microorganisms. The present study extends our knowledge of the interplay between these cells, bacteria and antibiotics in several respects. First, we have confirmed previous reports,[2,7] showing that unstimulated THP-1 cells are unable to control the intracellular multiplication of *L. monocytogenes* or *S. aureus*, and we extend this observation for *L. monocytogenes* to PMA-stimulated THP-1 cells. Secondly, we confirm that fluoroquinolones offer only limited protection against the intracellular forms of these organisms in unstimulated cells, with ~10% of the initial bacterial load apparently unaffected by the antibiotics within the time frame of the experiments (5 h) even though these drugs accumulate in the cells. Thirdly, we extend to moxifloxacin and to non-specific PMA activation our previous observation on the stimulatory effects exerted by interferon-γ on sparfloxacin accumulation and activity towards *L. monocytogenes* in THP-1 cells.[3] Yet, and quite surprisingly, this is not observed for levofloxacin, suggesting some sort of specific, differential recognition between drugs. Cell accumulation of fluoroquinolones is dependent on a dynamic balance between influx, intracellular binding to still undefined constituents, and efflux, all of which vary between fluoroquinolones.[8] Our data call for further studies examining these parameters and for systematic comparisons of molecules to allow us to draw meaningful structure–activity relationships. As PMA is a protein kinase C activator, we may also speculate that up-regulation of an inward transporter could be involved, as suggested for ciprofloxacin in polymorphonuclear leucocytes.[9] The fourth, and most unanticipated observation, is that the increase in the moxifloxacin cell accumulation results in an increased activity against *L. monocytogenes*, but not against *S. aureus*. This clearly reinforces our general conclusion that the intracellular activity of an antibiotic cannot be directly deduced from the level of its cellular accumulation only.[1] The most straightforward interpretation of the present data is that the increased accumulation of moxifloxacin in PMA-differentiated THP-1 cells probably exclusively affects its cytosolic component, as already observed for ciprofloxacin in murine macrophages when its cell accumulation increased by exposure to efflux inhibitors.[5] It is also possible that the effect exerted by acidic pH on moxifloxacin activity makes its increased accumulation ineffective towards *S. aureus*, which survives and thrives in the acidic phagolysosomal environment.

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Transparency declarations

None to declare.

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