JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 735–741

Received August 8, 2000
Accepted December 12, 2000
ISSN 0022-3573

Uptake of quinolones by in-vitro human monocyte derived macrophages

Martine Dorian, Jean Grellet and Marie C. Saux

Abstract

We have developed an in-vitro model of monocyte-derived macrophage (MDMØ) to compare fluoroquinolone uptake in monocytes and derived macrophages. Monocyte-derived macrophages were obtained in-vitro by cultivating freshly isolated monocytes for seven days in RPMI 1640 medium, containing foetal calf serum and Rhu granulocyte-macrophage colony stimulating factor. Final suspensions contained 95% viable cells and 63% macrophages. Intramacrophagic accumulation of ciprofloxacin, ofloxacin or sparfloxacin was measured at equilibrium after 30-min incubation in the presence of 16–18 μg mL^{-1} antibiotic. The results revealed low intra-cellular accumulation of ofloxacin in MDMØ (intracellular/extracellular ratio: IC/EC = 1.7). Ciprofloxacin and sparfloxacin uptake was significantly higher. The IC/EC ratios were only slightly increased in macrophages when compared with monocytes under the same experimental conditions. These results suggest that maturation of monocyte to macrophage has only a limited effect on basal quinolone uptake. Monocytic maturation cannot explain the important differences between fluoroquinolone accumulation in monocytes and tissue macrophages. Cell activation may be a greater determinant.

Introduction

Different pathogenic bacteria are able to survive and even to replicate inside phagocytic cells after they have been phagocytosed (Fenlon & Cynamon 1986). Persistence of these microorganisms may produce chronic or relapsing infections particularly in immunocompromised patients and the lung is a frequent site of such chronic intracellular infections. Eradication of intracellular pathogens requires the use of antibiotics active against these bacteria, which are able to concentrate within phagocytic cells and raise intracellular levels sufficient to kill these microorganisms (Cuffini et al 1992). Fluoroquinolones are potential antibiotics for the treatment of intracellular infections (Tulkens 1991) due to their important membrane diffusion and wide antimicrobial spectrum which includes obligate intracellular pathogens (*Listeria*, *Mycobacteria*) and facultative intracellular bacteria (*Legionella*, *Staphylococcus*). Fluoroquinolones are able to concentrate into phagocytic cells (Carlier et al 1987; Garcia et al 1992, 1994; Rispal et al 1996; Pascual et al 1999) but significant differences in accumulation exist between quinolones and also between phagocytic cell types. For example, ciprofloxacin, sparfloxacin and ofloxacin do not accumulate into monocytes freshly isolated from human blood (Dorian et al 1998) while quinolones accumulate in polymorphonuclear cells (Garcia et al 1992, 1994; Pascual et al 1989, 1997), tissue macrophages (Carlier et al 1987; Wise & Baldwin 1991; Schüler et al 1997) and macrophage-like cells (Rispal et al 1996). These

Laboratory of Pharmacokinetics and Clinical Pharmacy, University Victor Ségalen-Bordeaux II, Rue Leo Saignat 33076 Bordeaux Cedex, France

Martine Dorian, Jean Grellet, Marie C. Saux

Correspondence: J. Grellet, Laboratoire de Pharmacocinétique et Pharmacie Clinique, Université Victor Ségalen-Bordeaux II, 146 rue Léo Saignat, 33076 Bordeaux Cedex, France. E-mail: jean.grellet@chu-bordeaux.fr

Acknowledgement: We thank Dr P. Fialon and Dr F. Belloc for their constructive comments and Mr Ducint and A. Briais for their excellent technical assistance.

differences can be related to the mechanisms underlying fluoroquinolone uptake by human phagocytic cells (Steinberg 1994; Memin et al 1997; Pascual et al 1997). Sparfloxacin and trovafloxacin seem to use a passive mechanism to enter cells probably related to their high hydrophobicity, allowing for greater penetration of cell membranes (Garcia et al 1992; Pascual et al 1997). Other less hydrophobic quinolones such as ofloxacin, pefloxacin, moxifloxacin, Bay Y 3118 and ciprofloxacin seem to partially require an active process (Garcia et al 1994; Memin et al 1996, 1997; Pascual et al 1997, 1999).

The cellular differentiation and/or activation of phagocytic cells could be an important determinant of the quinolone accumulation by affecting the uptake mechanisms. Loo et al (1997) demonstrated that promyeloid HL 60 cells acquired an enhanced ability to take up ciprofloxacin as they matured to end stage polymorphonuclear cells. The same hypothesis may be applied to the maturation of monocytes to macrophages.

The aim of this study was to test the hypothesis that the uptake of quinolone antibiotics is quantitatively different between freshly isolated monocytes and in-vitro derived macrophages.

Materials and Methods

Monocytes collection

Peripheral blood monocytes were isolated from acid-citrate-dextrose venous blood of healthy donors. The isolation procedure described by Dorian et al (1998) was followed. This included a centrifugation on a lymphoprep gradient (d = 1.077) (Polylabo, Strasbourg, France) followed by a purification by specific adhesion and disadhesion of monocytes.

Briefly, mononuclear cells were collected at the plasma lymphoprep interphase, washed twice with Ca^{2+}/Mg^{2+}-free phosphate-buffered saline (free PBS) (Polylabo) and resuspended into RPMI 1640 medium (Polylabo) supplemented with 10% heat inactivated fetal calf serum (FCS). Cells were then layered on Petri dishes coated with fibronectin (Sigma, St Quentin Fallavier, France) for 15 min to allow specific adhesion of monocytes. Monocytes were released from the matrix by the action of a cold (0°C) 10 mM EDTA solution (Polylabo) for 15 min. Released monocytes were washed twice with free PBS and resuspended in RPMI 1640 medium containing 10% FCS to obtain a $3–4 \times 10^6$ cells mL^{-1} final suspension.

In-vitro monocyte-derived macrophages (MDMØ)

To obtain MDMØ, freshly isolated monocytes were cultured for seven days (cell density of 10^6 mL^{-1}) in RPMI 1640 medium supplemented with 10% FCS, 2 mM L-glutamine and 10 ng mL^{-1} recombinant human granulocyte/monocyte colony stimulating factor (Rhu GM-CSF) (Schering-Plough, Levallois Perret, France) but without any antibiotic.

Cultures were performed in Teflon bags (Polylabo); the hydrophobic inner surface prevents adhesion of cells. Cultures were maintained for seven days at 37°C in a humidified 5% CO_2 atmosphere. The RPMI 1640 medium remained unchanged during the cell culture period.

Characterization of monocytes and MDMØ

Monocytes, MDMØ and contaminant cells were characterized in cell suspensions by morphological examination of May-Grünwald-Giemsa-stained smears under light microscopy. Cell viability was determined using the trypan blue exclusion test to detect dead cells and acridine orange coloration to detect pre-apoptotic and apoptotic cells. Cells were considered viable when they excluded trypan blue and presented a normal aspect (green nuclei and regularly outlined red-orange cytoplasm) under fluorescent microscopy after staining for 1 min with a 100 μg mL^{-1} solution of acridine orange.

Immunophenotypes of cells in suspensions were determined immediately after monocyte collection (day 0) and after seven days culture (day 7) by double direct immunofluorescence using flow cytometry. Cells (10^6 mL^{-1}) were incubated for 30 min at room temperature (20°C) in presence of fluorescein isothiocyanate (FITC) or phycoerythrin (PE)-conjugated specific antibodies to detect CD 14, CD 45 and CD 71 cell surface antigens. Four monoclonal antibodies were used in combination: anti CD 14-FITC/anti CD 45-PE, anti CD 14-PE/anti CD 71-FITC (Coultronics, Margency, France) and polyclonal mouse immunoglobulins served as negative controls. Analyses were performed using a Coulter Epics XL flow cytometer. Cell populations were gated according to their forward and side scatter.

The cytochemical characteristics of cells were determined immediately after isolation (day 0) and after seven days (day 7) of culture. Myeloperoxidase intracellular activity was detected by microscopic examination after staining slides according to the technique of Kaplow (1965). The technique of Yam & Crosby (1971)

was used to detect alpha-naphtyl-naphtol-acetate esterase activity.

Quinolone uptake by monocyte and MDMØ

Uptake of ciprofloxacin, ofloxacin and sparfloxacin by monocyte and MDMØ was determined at equilibrium using the velocity gradient centrifugation technique (Rispal et al 1996). Fluoroquinolones were kindly supplied by Bayer Pharma (Sens, France) for ciprofloxacin, Rhone-Poulenc laboratory (Antony, France) for sparfloxacin and Diamant-Roussel laboratory (Romainville, France) for ofloxacin. In these experiments, freshly isolated monocytes or seven day cultures of MDMØ were incubated at a cell density of $3–4 \times 10^6$ mL^{-1} in polypropylene tubes at 37°C for 30 min in the presence of 16–18 μg mL^{-1} of the antibiotics under test.

The cells were separated from the extracellular solution by centrifugation through a water-impermeable silicone/mineral oil barrier into microcentrifuge tubes (Polylabo, 400-μL). The cells were disrupted at the bottom of the tubes by the action of a 1 M perchloric acid solution.

The fluoroquinolones were quantified in the extracellular incubation media and the perchloric lysates by high-performance liquid chromatography according to previously described techniques (Rispal et al 1996; Dorian et al 1998) using a Kontron model 400 chromatograph (Kontron, Velizy, France). Intracellular concentrations of the antibiotics were calculated by dividing the amount of drug present in the intracellular lysate by the total volume of monocyte or MDMØ calculated according to the following formula: $V = \Pi/6\, D^3 \times y10^6$, where $y10^6$ was the number of monocytes or MDMØ estimated in a 200-μL suspension. Monocytes or MDMØ in suspension were considered to be spherical and mean cell diameters (D) were estimated from 200 cells by microscopic examination. Total monocyte and MDMØ volumes were evaluated also in a series of three experiments from cellular protein contents according to the method proposed by Memin et al (1997). In these manipulations protein content was measured using the Lowry micromethod (Peterson 1977) (Sigma) and intracellular volumes (V) were calculated as $V = 11.5 \times P$, where P is the sample protein content.

Table 1 Different types of cells in suspensions just after isolation from blood (day 0) and after three and seven days of culture (characterization by morphological examination after May-Grünwald-Giemsa staining).

Day of culture	Lymphocytes (% of total cells)	Monocytes (% of total cells)	Macrophages (% of total cells)
Day 0 (n = 5)	23.4	74	2.4
Day 3 (n = 5)	17	57.2	25.8
Day 7 (n = 5)	18.8	18	63.2

Values are mean percentage of total cells, n = 5 experiments.

Statistical analysis

The data were expressed as the means ± s.d. Differences among groups were compared by analysis of variance, used to assess statistical significance at $P \leqslant 0.01$.

Results

Cell characteristics during in-vitro culture

Immediately after isolation cell viability was 98.4 ± 0.5%. Cells in suspensions were 74% monocytes and the other cells were lymphocytes (Table 1). The immunophenotypes of isolated cells (Table 2) allowed for the identification of two different cell populations. Cells of the first population strongly expressed the CD 45 antigen but not CD 14 and were mainly lymphocytes. The second population was composed for the most part of monocytes which expressed CD 14 and CD 45 surface antigens. Seventy-five percent of the isolated cells expressed a positive myeloperoxidase activity (Table 3). These cells were monocytes only.

Culture of isolated monocytes in the presence of FCS and Rhu GM-CSF was associated with a significant loss of cells. After seven days, the yield of cells recovered by approximately 40%. This loss affected the two populations of isolated cells (monocytes and lymphocytes) in a similar proportion. After three and seven days of culture 96.8 ± 2.6% and 95.6 ± 1.7%, respectively, of the remaining cells were viable according to trypan blue exclusion tests and acridine orange coloration.

The culture of unadherent monocytes in RPMI 1640 medium containing 10% FCS and 10 ng mL^{-1} Rhu GM-CSF resulted in a morphological change of monocytes (cytological examination) towards a macrophage phenotype. This shift started after three days of culture (Table 1) and at that time affected 35% of monocytes. At the end of the culture period (Table 1) 86% of initial monocytes presented a macrophage-like morphology.

The culture of isolated monocytes for seven days did not modify CD 14 and CD 45 expression, which remained high, but led to a significant increase in CD 71 expression (53.7 ± 14% vs 2.1 ± 1.1%; $P < 0.01$; Table

Table 2 Expression of cell surface antigens just after cell isolation (day 0) and after seven days of culture.

Day of culture	CD 14 antigen (%)		CD 45 antigen (%)		CD 71 antigen (%)	
	Lymphocytes	Monocytes/ macrophages	Lymphocytes	Monocytes/ macrophages	Lymphocytes	Monocytes/ macrophages
Day 0 (n = 5)	0	72.5 ± 13.8	98.8. ± 0.34	79.7 ± 13.4	0	2.1 ± 1.1
Day 7 (n = 5)	0	58 ± 14.9	ND	70.4 ± 17.2	0	53.7 ± 14*

Values are means ± s.d. percentages of each type cells, n = 5 experiments. *$P < 0.01$ compared with day 0. ND, Not determined.

Table 3 Cytochemical characteristics of monocyte/macrophage (MDMØ) just after cell isolation (day 0) and after seven days of culture.

Day of culture	% of cells showing myeloperoxidase activity	% of cells showing naphtyl-naphtol esterase activity
Day 0	75 ± 26 (n = 5)	3.25 ± 6 (n = 5)
Day 7	28.8 ± 28.1* (n = 5)	79.7 ± 25* (n = 5)

Values are means ± s.d. percentages of monocyte/MDMØ (identified by morphology). *$P < 0.01$ compared with day 0.

2). Peroxidase activity decreased during monocyte differentiation and after seven days of culture was present in only 28 % of cells. Inversely, the staining intensity of esterase granules increased significantly during monocyte maturation to macrophages with 80 % of the macrophages positive for non-specific esterase after seven days of culture (Table 3).

Uptake of quinolones by in-vitro monocyte-derived macrophages

Uptake of ciprofloxacin, ofloxacin and sparfloxacin was measured in monocytes and MDMØ at equilibrium after 30-min incubation at 37°C. Intracellular volumes calculated according to the formula $V = \Pi/6\ D^3 \times y10^6$ (1 μL for 10^6 monocytes and 2.48 μL for 10^6 MDMØ) were used to determine intracellular concentrations.

Accumulation of ofloxacin in monocytes was low at equilibrium since the intracellular/extracellular concentration (IC/EC) mean ratio was 1.30 (Table 4). Uptake of ciprofloxacin and sparfloxacin in monocytes was significantly higher ($P < 0.01$) compared with ofloxacin under the same conditions.

Accumulation of quinolones in MDMØ was 1.7 ± 0.7, 2.5 ± 0.5, and 3.2 ± 1.5 for ofloxacin, ciprofloxacin and sparfloxacin, respectively.

There was no statistically significant difference in the uptake of all three quinolones by macrophages and monocytes, although the uptake of ofloxacin was significantly lower in both cell types (Table 4).

Table 4 Uptake of ciprofloxacin, ofloxacin and sparfloxacin by in-vitro monocytes and macrophages at equilibrium.

	Ciprofloxacin	Ofloxacin	Sparfloxacin
Tested extracellular concn (μg mL^{-1}) of monocytes	17.0 ± 1.5	16.7 ± 1.5	16.9 ± 2.1
Tested extracellular concn (μg mL^{-1}) of macrophages (MDMØ)	16.3 ± 0.5	18 ± 1.4	18 ± 1.6
IC/EC ratio: monocytes	1.8 ± 0.2 (n = 5)	1.3 ± 0.45 (n = 5)	2.4 ± 0.3* (n = 5)
IC/EC ratio: macrophages (MDMØ)	2.5 ± 0.5* (n = 5)	1.7 ± 0.7 (n = 5)	3.2 ± 1.5* (n = 5)

Values are means ± s.d. *$P < 0.01$ compared with ofloxacin. IC/EC, intracellular/extracellular concentration.

Discussion

Monocyte-derived macrophages were obtained by in-vitro culture in hydrophobic Teflon bags, to avoid adhesion, in the presence of FCS, an essential constituent for cell survival and differentiation (Van der Meer et al 1982; Eischen et al 1992; Kreutz et al 1992). GM-CSF 10 ng mL^{-1} was added to the culture medium to limit apoptosis (Mangan & Wahl 1991) and to improve cell differentiation (Eischen et al 1992). Its concentration was chosen according to previously reported dose–response curves (Van der Meer et al 1982; Young et al 1990).

Such conditions provided non-adherent macrophagic cells and offered technical advantages, such as a possible separation of cells from the incubation media by velocity gradient centrifugation and mathematical calculation of cell volume based on a spherical cell shape.

In these in-vitro conditions, cultivation of blood monocytes induced morphological changes typical of mature macrophages. Maturation was accompanied by an increased expression of the transferrin receptor (CD 71) while culture had no significant influence on CD 14 expression. Such results are in accordance with previously reported data concerning in-vitro differentiation of monocytes (Van der Meer et al 1982; Eischen et al 1992; Kreutz et al 1992).

Maturation of cells was examined by analysing cytochemical markers. After seven days of culture, myeloperoxidase activity decreased markedly in monocyte-derived macrophages while naphtyl-naphtol esterase increased significantly. This shift in intracellular enzyme activity confirmed the differentiation of monocytes under these culture conditions (Andreesen et al 1983; Eischen et al 1992).

Little information is available concerning the yield of monocyte differentiation in-vitro. In this study, up to 80% of monocytes differentiated. As incomplete differentiation could impair the measurement of quinolone uptake in macrophages, we explored different possibilities to improve maturation. The prolongation of culture duration was rejected because it was associated with a decrease in cell survival. Moreover, after 10 days of culture Eischen et al (1992) reported changes in cell phenotype that could suggest functional alterations. The combination of GM-CSF and M-CSF (monocyte stimulating factor) could improve the yield of differentiation but was not tested in this study because Eischen et al (1992) showed that this combination induced different macrophages in terms of phenotypes.

Finally, the intracellular uptake of quinolones was measured on incompletely differentiated suspensions of cells and in such conditions the IC/EC ratios were relatively low (between 1.7 and 3.2). Methodological topics have to be discussed before interpreting these results.

Firstly, the extracellular fluoroquinolone concentration tested (16–18 $\mu g\ mL^{-1}$) might appear high when compared with in-vivo plasma levels. In fact, these concentrations were chosen to mimic tissue levels of fluoroquinolones in lung, particularly in the epithelial lining fluid, the normal environment of alveolar macrophages (Bergogne-Berezin 1992; Schüler et al 1997).

Secondly, the calculation of intracellular concentrations required reliable measurements of intracellular volumes. Classically, intracellular volumes are measured using radioactive tracers (Schüler et al 1997). In this study tritiated water and [^{14}C]inulin had been used but the results were unreliable; this could be due to a possible binding of inulin to the cell membrane. So, a mathematical formula was used to calculate intracellular volumes. This method appeared convenient since the volumes calculated for 10^6 monocytes (1 μL) and 10^6 macrophages (2.48 μL) were very close to previously reported values (Crapo et al 1982; Schüler et al 1997). Moreover, indirect measurements of intracellular volumes performed according to the method proposed by Memin et al (1996) from intracellular protein contents (0.87 μL for 10^6 monocytes and 2.83 μL for 10^6 MDMØ) correlated well.

Thirdly, intracellular uptake of quinolones by macrophages was measured using suspensions containing contaminant cells. As a consequence, quinolone uptake may have been underestimated. However, in all experiments the intracellular volumes of contaminant cells represented less than 15% of total cell volume. So, we can conclude that in-vitro uptake of quinolones by MDMØ remained relatively low when compared with tissue macrophages (Carlier et al 1987; Schüler et al 1997).

In these experiments, the accumulation of fluoroquinolones into macrophages was not significantly increased when compared with freshly isolated monocytes incubated under the same conditions. So, the differentiation of monocytes under these culture conditions had only a limited effect on basal quinolone uptake. Nevertheless, different data suggest that differentiation might enhance the ability of these cells to accumulate fluoroquinolones (Memin et al 1996, 1997). Loo et al (1997) reported that the maturation of human promyelocytic HL 60 cells increased their ability to accumulate ciprofloxacin in response to protein kinase C activation by phorbol myristate acetate. The important differences

between quinolone uptake by in-vitro MDMØ and tissue macrophages may be the consequence of differences in extracellular environment. Cell environment appears to influence antibiotic uptake and if phagocytosis of opsonized bacteria has no significant influence, other environmental factors may participate in increasing (Garcia et al 1992) or decreasing (Garcia et al 1994; Geertsma et al 1997) antibiotic accumulation.

In-vitro MDMØ were cultured in experimental conditions very different from the physiological environment. In particular, MDMØ were probably poorly activated (Thomassen et al 1989) and this may explain the low quinolone uptake in these cells.

Further investigations are required to confirm the increased ability of derived macrophages to accumulate quinolones in response to protein kinase C stimulation and to evaluate the effects of different activating factors.

References

Andreesen, R., Osterholtz, J., Bross, K. J., Schultz, A., Luckenbach, G. A., Löhr, G. W. (1983) Cytotoxic effector cell function at different stages of human monocyte-macrophage maturation. *Cancer Res*. **43**: 5931–5936

Bergogne-Berezin, E. (1992) Pharmacocinétique des antibiotiques dans les voies respiratoires. Limites de l'apport de cette information. *Med. Mal. Infect*. **22** (Special): 103–113

Carlier, M. B., Scorneaux, B., Zenebergh, A. (1987) Uptake and subcellular distribution of 4-quinolones (pefloxacin, ciprofloxacin, ofloxacin, R0 23–6240) in phagocytes. In: Program and Abstracts of the 27th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, New York, abstr. 622, p 205

Crapo, J. D., Barry, B. E., Gehr, P. (1982) Cell number and cell characteristics of the normal human lung. *Am. Rev. Respir. Dis*. **125**: 332–337

Cuffini, A. M., Tullio, V., Fazari, S., Allocco, A., Carlone, N. A. (1992) Pefloxacin and immunity: cellular uptake, potentiation of macrophage phagocytosis and intracellular bioactivity for *K Pneumoniae*. *Int. J. Tiss. React*. **14**: 131–139

Dorian, M., Grellet, J., Saux, M. C. (1998) Uptake of fluoroquinolones in human monocytes isolated from peripheral blood. *J. Pharm. Pharmacol*. **50**: 783–788

Eischen, A., Vincent, F., Louis, B. (1992) Immunophenotypic characterization of human peritoneal and alveolar macrophages and of human blood monocytes differentiated in the presence of either GM-CSF or M-CSF or a combination of GM-CSF/M-CSF. *Nouv. Rev. Fr. Hematol*. **34**: 421–434

Fenlon, C. H., Cynamon, M. H. (1986) Comparative in-vitro activities of ciprofloxacin and other 4-quinolones against *Mycobacterium tuberculosis* and *Mycobacterium intracellulare*. *Antimicrob. Agents Chemother*. **29**: 386–388

Garcia, I., Pascual, A., Guzman, M. C. (1992) Uptake and intracellular activity of sparfloxacin in human polymorphonuclear leukocytes and tissue culture cells. *Antimicrob. Agents Chemother*. **36**: 1053–1056

Garcia, I., Pascual, A., Perea, E. J. (1994) Intracellular penetration and activity of BAY Y 3118 in human PMN leukocytes. *Antimicrob. Agents Chemother*. **38**: 2426–2429

Geertsma, M. F., Van Furth, R., Nibbering, P. H. (1997) Monocytes incubated with surfactant: a model for human alveolar macrophages? *J. Leuk. Biol*. **62**: 485–492

Kaplow, L. S. (1965) Simplified myeloperoxidase stain using benzidine dihydrochloride. *Blood* **26**: 215–219

Kreutz, M., Krause, S. W., Hennemann, B., Rehm, A., Andreesen, R. (1992) Macrophage heterogeneity and differentiation: defined serum-free culture conditions induce different types of macrophages in-vitro. *Res. Immunol*. **143**: 107–115

Loo, K. C., Cario, A. C., Zhang, F., Walters, J. D. (1997) Regulation of ciprofloxacin uptake in human promyelocytic leukemia cells and polymorphonuclear leukocytes. *J. Leuk. Biol*. **61**: 619–623

Mangan, D. F., Wahl, S. M. (1991) Differential regulation of human monocyte programmed cell death (apoptosis) by chemotactic factors and pro-inflammatory cytokines. *J. Immunol*. **147**: 3408–3412

Memin, E., Panteix, G., Revol, A. (1996) Is the uptake of pefloxacin in human blood monocytes a simple diffusion process? *J. Antimicrob. Chemother*. **38**: 787–798

Memin, E., Panteix, G., Revol, A. (1997) Carrier mediated system for pefloxacin uptake in human monocytes. *J. Antimicrob. Chemother*. **40**: 263–268

Pascual, A., Garcia, I., Perea, E. J. (1989) Fluorometric measurement of ofloxacin uptake by human polymorphonuclear leukocytes. *Antimicrob. Agents Chemother*. **33**: 653–656

Pascual, A., Garcia, I., Ballesta, S., Perea, E. J. (1997) Uptake and intracellular activity of trovafloxacin in human phagocytes and tissue-cultured epithelial cells. *Antimicrob. Agents Chemother*. **41**: 274–277

Pascual, A., Garcia, I., Ballesta, S., Perea, E. J. (1999) Uptake and intracellular activity of moxifloxacin in human neutrophils and tissue-cultured epithelial cells. *Antimicrob. Agents Chemother*. **43**: 12–15

Peterson, G. L. (1977) A simplification of the protein assay method of Lowry et al which is more generally applicable. *Anal. Biochem*. **83**: 346–356

Rispal, P., Grellet, J., Celerier, C., Breihl, D., Dorian, M., Pellegrin, J. L., Saux, M. C., Leng, B. (1996) Comparative uptake of sparfloxacin and ciprofloxacin into human THP1 monocytic cells. *Arzneimittelforschung Drug Res*. **46**: 316–319

Schüler, P., Zemper, K., Borner, K., Koeppe, P., Schaberg, T., Lode, H. (1997) Penetration of sparfloxacin and ciprofloxacin into alveolar macrophages, ELF and PMN leukocytes. *Eur. Respir. J*. **10**: 1130–1136

Steinberg, T. H. (1994) Cellular transport of drugs. *Clin. Infect. Dis*. **19**: 916–921

Thomassen, M. J., Barna, B. P., Rankin, D., Wiedermann, H. P., Ahmad, M. (1989) Differential effect of recombinant GM-CSF on human monocytes and alveolar macrophages. *Cancer Res*. **49**: 4086–4089

Tulkens, P. M. (1991) Intracellular distribution and activity of antibiotics. *Eur. J. Clin. Microbiol. Infect. Dis*. **10**: 100–106

Van Der Meer, J. W. M., Van De Gevel, J. S., Blusse Van Oud Alblas, A., Kramps, J. A., Van Zwet, T. L., Leijh, P. C. J., Van Furth, R.

(1982) Characteristics of human monocytes cultured in the Teflon culture bag. *Immunology* **47**: 617–625

Wise, R., Baldwin, D. R. (1991) Comparative pharmacokinetics disposition of fluoroquinolones in the lung. *J. Antimicrob. Chemother.* **28** (Suppl. C): 65–71

Yam, L. T., Li, C. Y., Crosby, W. H. (1971) Cytochemical identification of monocytes and granulocytes. *Am. J. Clin. Pathol.* **55**: 283–290

Young, D. A., Lowe, L. D., Clarck, S. C. (1990) Comparison of the effects of IL3, GM-CSF and M-CSF in supporting monocyte differentiation in culture. Analysis of macrophage antibody-dependant cellular cytotoxicity. *J. Immunol.* **145**: 607–615