Journal of Antimicrobial Chemotherapy (2006) **57**, 230–235
doi:10.1093/jac/dki441
Advance Access publication 13 December 2005

JAC

Anti-inflammatory effects of moxifloxacin on IL-8, IL-1β and TNF-α secretion and NFκB and MAP-kinase activation in human monocytes stimulated with *Aspergillus fumigatus*

Itamar Shalit*, Drora Halperin, Debby Haite, Avital Levitov, Jacob Romano, Nir Osherov and Ina Fabian

Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel

Received 8 April 2005; returned 15 September 2005; revised 1 October 2005; accepted 7 November 2005

Objectives: **We have previously shown that moxifloxacin conferred protective anti-inflammatory effects against *Candida* pneumonia in immunosuppressed mice. Further *in vitro* studies showed anti-inflammatory effects of moxifloxacin in LPS and cytokine-stimulated monocytic and epithelial cells. In the present study, concentrating on a more challenging pathogen of immunosuppressed hosts, we studied the effect of moxifloxacin on cytokine secretion and signal transduction mechanisms in monocytic cells stimulated with *Aspergillus fumigatus.***

Methods: **Human peripheral blood monocytes (PBMCs) and a human monocytic cell line (THP-1) were incubated with 1.5×10^6/mL conidia of a clinical isolate of *A. fumigatus*. Cytokine secretion and activation of NFκB and the MAP-kinases ERK1/2 and p38 were measured with and without the addition of moxifloxacin (5–20 mg/L).**

Results: **Stimulation of PBMCs and THP-1 cells with *A. fumigatus* increased IL-8, IL-1β and TNF-α secretion (4.1-, 8.3- and 7-fold, and 5.4-, 3.7- and 17.8-fold, respectively). Addition of moxifloxacin (5–20 mg/L) inhibited cytokine secretion up to 45.7 ± 5%, 72 ± 13% and 73 ± 10% in PBMCs and up to 35.6 ± 0.5%, 30 ± 2.4% and 19 ± 4% in THP-1 cells ($P < 0.05$). Signal transduction studies showed that incubation of THP-1 cells with *A. fumigatus* increased ERK1/2 and p38 phosphorylation and p65-NFκB protein expression by 1.6-, 1.3- and 1.8-fold, respectively. Addition of moxifloxacin inhibited ERK1/2, p38 and p65-NFκB by up to 69 ± 14%, 58 ± 3% and 75 ± 15%, respectively.**

Conclusions: **Our results indicate that moxifloxacin acts as an anti-inflammatory agent in monocytic cells stimulated with *A. fumigatus* conidia. Whether these effects may be protective as in the *Candida* pneumonia model is unknown and merits *in vivo* studies in models of pulmonary aspergillosis.**

Keywords: cytokines, aspergillosis, immunomodulation, *A. fumigatus*

Introduction

Invasive aspergillosis is one of the most common invasive fungal infections in immunocompromised hosts. It carries an overall case fatality rate of 58% with >80% mortality in bone marrow transplant recipients.[1] Mortality remains high despite the introduction of new antifungal agents of the azole and echinocandin groups[2,3] and highlights the need for new therapeutic modalities. We have previously shown that the fluoroquinolone moxifloxacin conferred protective anti-inflammatory effects in a murine model of *Candida* pneumonia in immunosuppressed animals, resulting in a marked decrease in bronchopneumonia and enhanced survival.[4] The protective efficacy was associated with significant reduction in IL-8 and TNF-α in lung homogenates as well as significant inhibition in NFκB nuclear translocation into alveolar macrophages and epithelial cells demonstrated by immunohistochemistry.[5] Further *in vitro* studies showed anti-inflammatory effects of moxifloxacin in LPS and cytokine-stimulated human monocytic cells and enabled us to elucidate the specific inhibitory effects of the drug on key signal transduction pathways associated

*Correspondence address. Paediatric Infectious Diseases Unit, Schneider Children's Medical Centre of Israel, 14 Kaplan Street, Petach-Tikvah 49202, Israel. Tel: +972-3-9253206; Fax: +972-3-9247515; E-mail: shalit_i@netvision.net.il

© The Author 2005. Published by Oxford University Press on behalf of the British Society for Antimicrobial Chemotherapy. All rights reserved. For Permissions, please e-mail: journals.permissions@oxfordjournals.org

with pro-inflammatory responses, the NFκB and the MAP-kinases pathways.[6]

Previous studies have shown that various cells of macrophage lineage are affected by *Aspergillus* conidia *in vitro*[7] and *in vivo*[8] and take an active part in the inflammatory process associated with pulmonary aspergillosis. In the present study we aimed to extend our previous observations to the more clinically challenging pathogen for immunosuppressed patients, and to study the effect of moxifloxacin on pro-inflammatory cytokines and signal transduction pathways in human monocytic cells stimulated with a clinical isolate of *A. fumigatus*.

Materials and methods

Human monocytes and THP-1 cells

Peripheral blood was drawn from healthy volunteers. Peripheral blood mononuclear cells were isolated by centrifugation on Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) and were suspended in RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum (FBS) (Hyclone Laboratories, Logan, UT, USA), 2 mM L-glutamine, 100 U/mL of penicillin and 100 µg/mL of streptomycin. Peripheral blood mononuclear cells were then plated and incubated for 1 h at 37°C in a humidified 95% air/5% CO_2 atmosphere. Non-adherent cells were removed by washing with PBS. More than 90% of the adherent cells were morphologically identified as monocytes, ~7% were lymphocytes and 2% were neutrophils. The viability of the cells was >98% as determined by Trypan Blue staining.

The human monocytic THP-1 cells (ATCC TIB 202) were maintained in RPMI medium supplemented with 10% heat inactivated FBS, 2 mM L-glutamine, 100 U/mL of penicillin and 100 µg/mL of streptomycin at 37°C in a humidified incubator with 5% CO_2. Assays were performed at a density of 1×10^6 cells/mL.

Preparation of Aspergillus

A. fumigatus (AF293) was grown on YAG solid medium (0.5% w/v yeast extract, 1% dextrose and 1.5% agar), for 2 days at 37°C, until conidia were mature. Mature conidia were collected by scraping the plates with 5 mL of 0.2% Tween 80 in sterile distilled water and washed three times in PBS as previously described.[9] Conidia were counted and re-suspended in PBS at a final concentration of 1–2 × 10^8 conidia/mL. Conidia were routinely used for all experiments after 2 days of harvesting.

IL-8, IL-1β and TNF-α production assays by ELISA

THP-1 cells (1×10^6/mL) and human peripheral blood monocytes (PBMCs) (5×10^5/mL) were suspended in RPMI medium supplemented with 2% human serum or 5% autologous serum, respectively, and placed in 35 mm culture plates. The cells were stimulated with conidia of a clinical isolate of *A. fumigatus* (1.5×10^6/mL) for 8 and 6 h, respectively, in the absence or presence of moxifloxacin (2.5, 5, 10 and 20 mg/L) (Bayer AG, Wuppertal, Germany). Cell-free supernatants were recovered by centrifugation and stored at –20°C until assayed. The concentrations of IL-8, TNF-α and IL-1β were determined using ELISA (R&D Systems, Inc., Minneapolis, MN, USA). The sensitivity of the assay for IL-8 was >10 pg/mL, for IL-1β >4 pg/mL and for TNF-α >15 pg/mL.

Western-blot analysis of ERK and p38

THP-1 cells stimulated with *A. fumigatus* were incubated with 5, 10 and 20 mg/L of moxifloxacin for 0–120 min. Following incubation the cells were collected on ice, washed twice with ice-cold PBS and suspended in 40 µL of the lysis buffer: 50 mM Tris pH 7.6, 150 mM NaCl, 5 mM EDTA pH 8.0, 0.6% NP-40, 1 mM Na_3VO_4, 20 mM β-glycerophosphate, 1 mM phenylmethylsulphonyl fluoride, 2 mM *p*-nitrophenyl phosphate and 1:25 Complete Mini Protease Inhibitor Cocktail (Boehringer Mannheim, Germany). After being kept on ice for 15 min the lysates were subjected to centrifugation (20 000 **g**) at 4°C for 15 min to obtain a cytosolic fraction. The protein concentration was determined by a Bradford assay (Bio-Rad, Munich, Germany) before storage at –70°C. An aliquot of the cytosol fraction containing 35 µg of protein was resolved by 10% SDS–PAGE. After electrophoresis and electrophoretic transfer of proteins to nitrocellulose membranes (Schleicher and Shuell, Dassel, Germany), the membranes were blocked with 3% non-fat milk in Tris-buffered saline (pH 7.4) containing 0.1% Tween (TBST) for 1 h. Membranes were then rinsed three times in TBST and incubated at room temperature (RT) with mouse monoclonal anti-MAP-kinase, activated (di-phosphorylated) ERK1/2 Ab (1:20 000), monoclonal anti-MAP-kinase, non-phosphorylated ERK Ab (Sigma Chemical Co., St Louis, MO, USA) (1:3000 dilution), anti-phospho-p38 MAP-kinase Ab and p38 MAP-kinase Ab (1:1000 dilution, each Ab) (Cell Signaling Technology, Beverly, MA, USA). The blots were then incubated with a secondary antibody, horseradish peroxidase-linked anti-mouse IgG (Santa Cruz Biotechnology), for 1 h at RT. The blots were washed three times in TBST and incubated in enhanced chemiluminescence reagent (ECL, Amersham Pharmacia Biotech). Relative density values of ERK1/2 and p38 were determined by densitometric analysis followed by photographing the specific bands (Kodak XLS-1 film).

Western-blot analysis of NFκB

Nuclear extracts used in the western-blot analyses were prepared for THP-1 cells as previously described by us.[6] An aliquot of the nuclear fraction containing 50 µg of protein for NFκB was resolved by 10% SDS–PAGE as described above. NFκB was detected by incubating blots with anti-NFκB p65 rabbit polyclonal Ab (1 : 500 dilution) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Actin levels were also assessed as a loading control using an antibody (Santa Cruz Biotechnology) that reacts with a broad range of actin isoforms. The blots were then incubated with a secondary antibody, horseradish peroxidase-linked anti-rabbit IgG (Santa Cruz Biotechnology). After 1 h at RT and three washes in TBST, blots were incubated in ECL (Amersham Pharmacia Biotech).

Statistics

Statistical significance was determined by using the Student's paired *t*-test. Results were considered significant for P values < 0.05.

Results

The results are summarized in Figures 1–4. Exposure of THP-1 cells to *A. fumigatus* resulted in a 5.4-, 3.7- and 17.8-fold increase in the secretion of IL-8, IL-1β and TNF-α, respectively, compared with untreated cells (Figure 1a). The addition of moxifloxacin at concentrations of 5, 10 and 20 mg/L to *A. fumigatus*-stimulated THP-1 cells inhibited the secretion of IL-8 (by 19.7 ± 1.8%, 28.2 ± 0.5% and 35.6 ± 0.5%, respectively) ($P < 0.05$), IL-1β (by 22 ± 2%, 24.5 ± 4% and 30 ± 2.4%, respectively) ($P < 0.05$) and TNF-α (by 19 ± 4%, 12 ± 0.8% and 12.5 ± 2.7%, respectively) ($P < 0.05$) (Figure 1b).

Exposure of PBMCs to *A. fumigatus* resulted in a 4.1-, 8.3- and 7-fold increase in the secretion of IL-8, IL-1β and TNF-α,

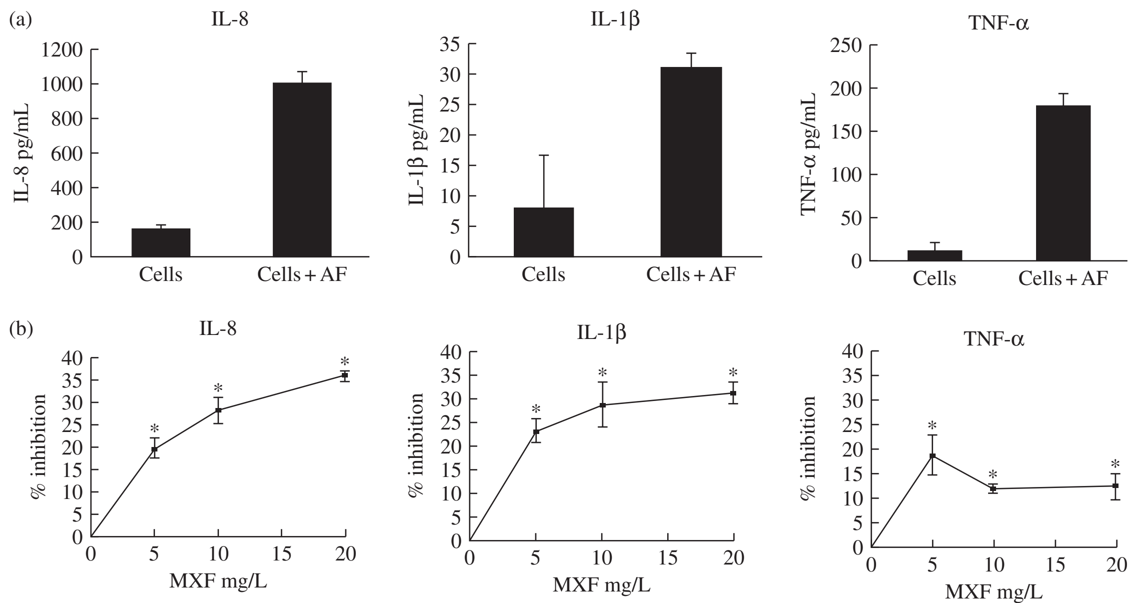

Figure 1. Production of IL-8, IL-β and TNF-α by *A. fumigatus* (AF)-stimulated THP-1 cells and the effect of moxifloxacin (MXF). THP-1 cells were stimulated with 1.5×10^6/mL AF for 8 h in the absence (a) or presence (b) of 5–20 mg/L MXF. The concentrations of IL-8, IL-β and TNF-α in the culture supernatant were measured by ELISA. Results are expressed as means ± SEM of three experiments.*$P < 0.05$ compared with no MXF.

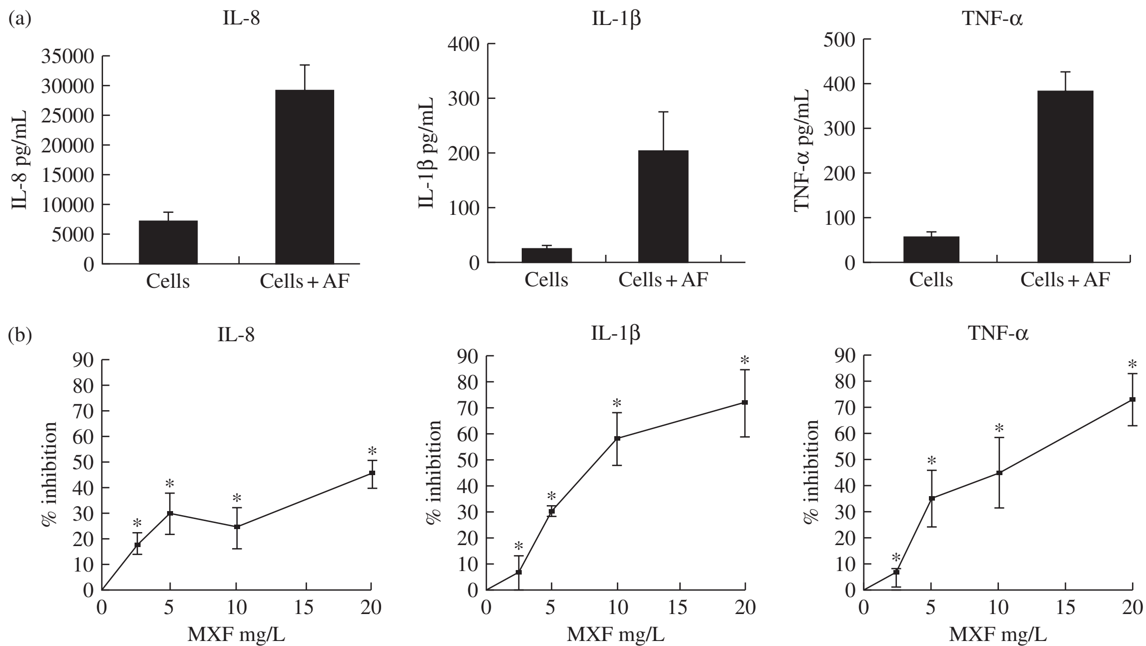

Figure 2. Production of IL-8, IL-β and TNF-α by *A. fumigatus* (AF)-stimulated PBMCs and the effect of moxifloxacin (MXF). PBMCs were stimulated with 1.5×10^6/mL AF for 6 h in the absence (a) or presence (b) of 5–20 mg/L MXF. The concentrations of IL-8, IL-β and TNF-α in the culture supernatant were measured by ELISA. Results are expressed as means ± SEM of three experiments. *$P < 0.05$ compared with no MXF.

respectively, compared with untreated cells (Figure 2a). The addition of moxifloxacin (5–20 mg/L) inhibited, in a dose-dependent manner, the secretion of IL-8, IL-1β and TNF-α up to 45.7 ± 5%, 72 ± 13% and 73 ± 10%, respectively ($P < 0.05$ for all moxifloxacin concentrations) (Figure 2b).

The results of ERK1/2 and p38 activation by *A. fumigatus* and the effect of moxifloxacin on the activation are presented in Figure 3. *A. fumigatus* induced a transient increase in ERK1/2 and p38 activities, which peaked at 15 min and declined at 120 min (Figure 3a).

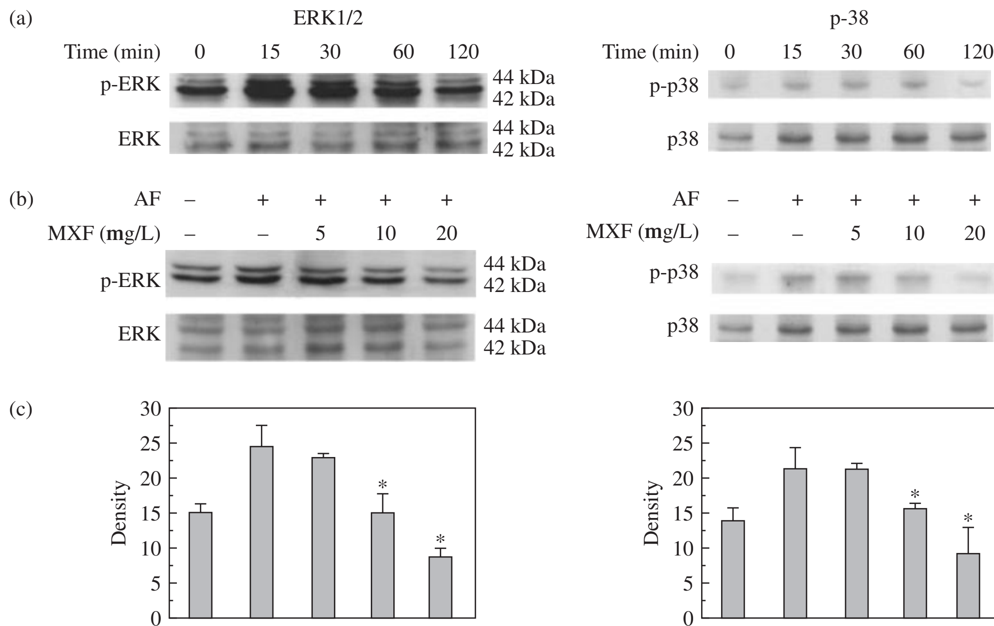

Figure 3. Activation of ERK1/2 and p38 in *A. fumigatus* (AF)-stimulated THP-1 cells and the effect of MXF. (a) THP-1 cells were incubated with AF for the indicated times. Cytoplasmic extracts were prepared. Western blots of ERK1/2 and p38 were performed as described in the Materials and methods section. (b) Effect of MXF: various concentrations of MXF were added (as indicated in the figure) and THP-1 cells were stimulated with AF for 15 min before performing western-blot analysis. The representative blots of three independent experiments are shown. (c) The densitometric analyses of western blots from three experiments are shown (means ± SEM). *$P < 0.05$.

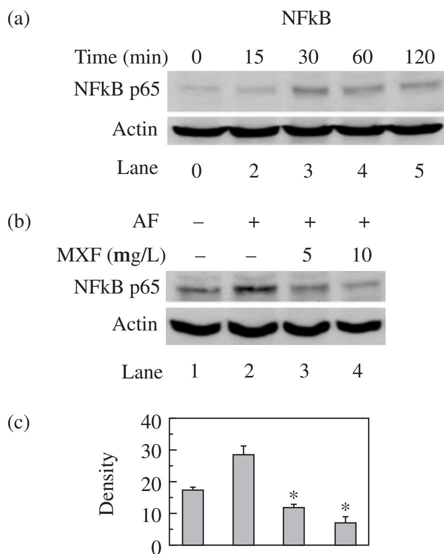

Figure 4. Expression of p65 protein of NFκB in *A. fumigatus* (AF)-stimulated THP-1 cells and the effect of MXF. (a) THP-1 cells were incubated with AF for the indicated times. Nuclear extracts were prepared. Western blot of the p65 protein was performed as described in the Materials and methods section. (b) Effect of MXF: various concentrations of MXF were added (as indicated in the figure) and THP-1 cells were stimulated with AF for 30 min before performing western-blot analysis. The representative blots of three independent experiments are shown. (c) The densitometric analyses of western blots from three experiments are shown (mean ± SEM). *$P < 0.05$.

Figure 3 (b and c) shows that addition of moxifloxacin inhibited *A. fumigatus*-induced ERK1/2 phosphorylation by 43 ± 8% and 69 ± 14% at 10 and 20 mg/L and p38 phosphorylation by 28 ± 1.4% and 58 ± 3%, respectively.

A. fumigatus significantly induced NFκB nuclear translocation, in a time-dependent manner, peaking at 30 min and slightly declining at 60 min (Figure 4a). Figure 4 (b and c) shows that addition of moxifloxacin decreased *A. fumigatus*-induced p65 NFκB expression by 60 ± 6% and 75 ± 15% at 5 and 10 mg/L.

Discussion

Our study demonstrates, in agreement with other reports, a pronounced pro-inflammatory response of human monocytic cells following exposure to *A. fumigatus*.[10] This response is mediated, among others, by the Toll-like receptors 2 and 4 via the NFκB signal transduction cascade[11] and the MAP-kinases ERK and p38, as demonstrated in our present study.

The pronounced pro-inflammatory response to *A. fumigatus* is linked both to protection against infection but at the same time to the pathogenesis of invasive aspergillosis.[12–14] Warris *et al.*[15] have demonstrated a significantly higher pro-inflammatory cytokine secretion in whole blood cells obtained from chronic granulomatous disease (CGD) patients that were exposed to *A. fumigatus*, as compared with cells obtained from healthy volunteers. They suggested that the higher release of pro-inflammatory cytokines in CGD patients may indicate that a dysregulation between pro- and anti-inflammatory responses contributes to the increased susceptibility to invasive aspergillosis in this patient group.

Our results indicate that moxifloxacin modulates the *A. fumigatus*-induced inflammatory response and induces a significant anti-inflammatory effect in the *A. fumigatus*-stimulated monocytes. The anti-inflammatory effects are demonstrated by IL-8, IL-1β and TNF-α inhibition and are further shown by

moxifloxacin inhibition of the NFκB and the MAP-kinases ERK and p38 pathways in the *A. fumigatus*-stimulated monocytes. It should be noted that the above effects were achieved with moxifloxacin concentrations that are clinically relevant, especially for lung-associated infections. Thus, peak plasma concentrations of moxifloxacin after single and repeated 400 mg oral doses reach 3–5 mg/L, whereas much higher concentrations are achieved in lung epithelial lining fluid (20.7 mg/L) and alveolar macrophages (56.7 mg/L) as measured 2.2 h following a single 400 mg oral dose of moxifloxacin in patients undergoing fibre-optic bronchoscopy.[16]

Several studies have demonstrated the ability of certain quinolones to confer protective anti-inflammatory effects (reviewed in ref. 17). In two studies by Khan *et al*.[18] and Purswani *et al*.,[19] ciprofloxacin and trovafloxacin protected mice from lethal and sub-lethal LPS injection, resulting in significant decreased TNF-α, IL-6 and IL-12 levels in serum and increased survival. In another study by Breban *et al*.,[20] ciprofloxacin exerted preventive clinical and histological effects against type II collagen-induced arthritis in rats. Moxifloxacin was shown to have anti-inflammatory effects in human umbilical vein endothelial cells infected with *Chlamydia pneumoniae* or stimulated with TNF-α leading to a decrease in neutrophil and monocyte trans-endothelial migration as well as a decrease in endothelial secretion of IL-8 and monocyte chemotactic protein 1.[21]

In our previous study we demonstrated a protective anti-inflammatory effect of pre-treatment with moxifloxacin in immunosuppressed mice challenged intratracheally with *Candida albicans*.[4] The significant protection against development of severe bronchopneumonia conferred by moxifloxacin was associated with a marked decrease in IL-8 and TNF-α secretion from the animals' lungs as well as inhibition of NFκB in alveolar macrophages and epithelial cells.[5] This effect was further studied *in vitro* and showed the same anti-inflammatory effects in human monocytic cells, and further elucidated the inhibitory effect of moxifloxacin on the NFκB and MAP-kinase signal transduction pathways.[6] Our present findings demonstrating similar significant anti-inflammatory effects of moxifloxacin in *A. fumigatus*-stimulated human monocytes, may imply a potential protective efficacy of moxifloxacin against *A. fumigatus* infection in immunosuppressed hosts. Based on our *in vivo Candida* study and the present results it is plausible that prophylactic use of moxifloxacin in patients undergoing chemotherapy or immunosuppression may confer protection against certain fungal infections. However, this consideration may raise the question of the potential risks of prophylactic treatment with quinolones in patients receiving chemotherapy, owing to development of resistant bacteria or enhanced superinfection rates. Two recent studies offer new insight into this specific issue.[22,23] In both randomized, double-blind, placebo-controlled studies the quinolone agent levofloxacin was administered prophylactically to patients undergoing chemotherapy, who were expected to have severe neutropenia. In both studies the prophylactic regimen was significantly beneficial and led to a substantial reduction in febrile episodes requiring empirical antimicrobial therapy, the rates of probable infections[22,23] as well as in the number of microbiologically documented infections, including bacteraemias owing to a single Gram-negative isolate and polymicrobial bacteraemias.[22] Although the rate of levofloxacin-resistant bacteria was 3% as compared with 1% in the placebo group this had no bearing on any clinical outcome measure and was considered by the authors as not clinically significant and possibly of transient and reversible nature.[22] Since moxifloxacin and levofloxacin have very similar spectra of antibacterial activity and clinical indications it can be assumed that short-term prophylactic use with moxifloxacin would not be associated with any increased risks as compared with levofloxacin. The above considerations coupled with our current and previous studies merit further studies on the potential protective antifungal effects of moxifloxacin in animal models of invasive aspergillosis and in clinical settings.

Acknowledgements

I. S. and I. F. are recipients of research grants from Bayer AG, Germany. N. O. and I. S. are recipients of a research grant from Merck and Co., USA.

Part of this work was presented at the Forty-fourth Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, USA, October 2004.

Transparency declarations

None to declare.

References

1. Lin SJ, Schranz J, Teutsch SM. Aspergillosis case-fatality rate: systematic review of the literature. *Clin Infect Dis* 2001; **32**: 358–66.

2. Herbrecht R, Denning DW, Paterson TF *et al*. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis *N Engl J Med* 2002; **347**: 408–15.

3. Maertens J, Raad I, Petrikkos G *et al*. Efficacy and safety of caspofungin for treatment of invasive aspergillosis in patients refractory to or intolerant of conventional antifungal therapy. *Clin Infect Dis* 2004; **39**: 1536–71.

4. Shalit I, Horev-Azaria L, Fabian I *et al*. Immunomodulatory and protective effects of moxifloxacin against *Candida albicans*-induced bronchopneumonia in mice injected with cyclophosphamide. *Antimicrob Agents Chemother* 2002; **46**: 2442–9.

5. Blau H, Fabian I, Kletter Y *et al*. Protective immunomodulation by moxifloxacin against *Candida albicans* lung infection in cyclophosphamide treated mice: suppression of TNF-α, IL-8 and NF-kB activation. In: *Abstracts of the 2002 Meeting of the American Thoracic Society, Atlanta, GA, 2002.* Abstract B-59. American Thoracic Society, New York, NY, USA.

6. Weiss T, Shalit I, Blau H *et al*. Anti-inflammatory effects of moxifloxacin on activated human monocytic cells: inhibition of NF-κB and mitogen-activated protein kinase activation and of synthesis of proinflammatory cytokines. *Antimicrob Agents Chemother* 2004; **48**: 1974–82.

7. Nessa K, Jarstrand C, Johansson A *et al*. *In vitro* interaction of alveolar macrophages and *Aspergillus fumigatus*. *Environ Res* 1997; **74**: 54–60.

8. Chilvers ER, Spreadbury CL, Cohen J. Bronchoalveolar lavage in an immunosuppressed rabbit model of invasive aspergillosis. *Mycopathologia* 1989; **108**: 163–71.

9. Yekutiel A, Shalit I, Shadkhan Y *et al*. *In vitro* activity of caspofungin combined with sulfamethoxazole against clinical isolates of *Aspergillus* spp. *Antimicrob Agents Chemother* 2004; **48**: 3279–83.

10. Wang JE, Warris A, Ellingsen EA *et al*. Involvement of CD14 and Toll-like receptors in activation of human monocytes by *Aspergillus fumigatus* hyphae. *Infect Immun* 2001; **69**: 2402–6.

11. Mambula SS, Sau K, Henneke P *et al*. Toll-like receptor (TLR) signaling in response to *Aspergillus fumigatus*. *J Biol Chem* 2002; **277**: 39320–26.

12. Roilides E, Holmes A, Blake C *et al*. Antifungal activity of elutriated human monocytes against *Aspergillus fumigatus* hyphae: enhancement by granulocyte-macrophage colony-stimulating factor and interferon-γ. *J Infect Dis* 1994; **170**: 894–9.

13. Mehrad B, Strieter RM, Standiford TJ. Role of TNF-α in pulmonary host defense in murine invasive aspergillosis. *J Immunol* 1999; **162**: 1633–40.

14. Roilides E, Dimitriadou A, Kadiltsoglou I *et al*. IL-10 exerts suppressive and enhancing effects on anti-fungal activity of mononuclear phagocytes against *Aspergillus fumigatus*. *J Immunol* 1997; **158**: 322–9.

15. Warris A, Netea MG, Wang JE *et al*. Cytokine release in healthy donors and patients with chronic granulomatous disease upon stimulation with *Aspergillus fumigatus*. *Scand J Infect Dis* 2003; **35**: 482–7.

16. Soman A, Honeybourne D, Andrews J *et al*. Concentrations of moxifloxacin in serum and pulmonary compartments following a single 400 mg oral dose in patients undergoing fibre-optic bronchoscopy. *J Antimicrob Chemother* 1999; **44**: 835–8.

17. Dalhoff A, Shalit I. Immunomodulatory effects of quinolones. *Lancet Infect Dis* 2003; **3**: 359–71.

18. Khan AA, Slifer TR, Araujo FG *et al*. Protection against lipopolysaccharide-induced death by fluoroquinolones. *Antimicrob Agents Chemother* 2000; **44**: 3169–73.

19. Purswani MU, Eckert SJ, Arora HK *et al*. Effect of ciprofloxacin on lethal and sublethal challenge with endotoxin and on early cytokine response in a murine *in vivo* model. *J Antimicrob Chemother* 2002; **50**: 51–8.

20. Breban M, Fournier C, Gougerot-Pocidalo MA *et al*. Protective effects of ciprofloxacin against type II collagen induced arthritis in rats. *J Rheumatol* 1992; **19**: 216–22.

21. Uriarte SM, Molestina RE, Miller RD *et al*. Effects of fluoroquinolones on the migration of human phagocytes through *Chlamydia pneumoniae*-infected and tumor necrosis factor alpha-stimulated endothelial cells. *Antimicrob Agents Chemother* 2004; **48**: 2538–43.

22. Bucaneve G, Micozzi A, Menicheti F *et al*. Levofloxacin to prevent bacterial infection in patients with cancer and neutropenia. *N Engl J Med* 2005; **353**: 977–87.

23. Cullen M, Steven N, Billingham L *et al*. Antibacterial prophylaxis after chemotherapy for solid tumors and lymphomas. *N Engl J Med* 2005; **353**: 988–98.