Influence of fluoroquinolones on phagocytosis and killing of *Candida albicans* by human polymorphonuclear neutrophils

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*Candida albicans* infections often occur during or shortly after antibacterial treatment. Phagocytosis by polymorphonuclear neutrophil granulocytes (PMN) is the most important primarily defence mechanism against *C. albicans*. Certain antibiotics such as some fluoroquinolones (FQ) are known to influence phagocyte functions. Thus, we investigated the influence of older and newer FQ on the phagocytosis and killing of *C. albicans* by human PMN paying special attention to CD11b expression of these cells as an indicator of the degree of their activation. In order to obtain comprehensive and comparable results we tested 13 FQ over a wide range of concentrations and in a time dependent manner in a standardized approach. When used at therapeutic concentrations, the FQ tested did not influence to a clinically significant degree the phagocytosis or the killing of *C. albicans* by human PMN and also not their activation. However, at high concentrations those FQ with cyclopropyl-moiety at position N1 showed increase in CD11b expression and diminished phagocytosis and oxidative burst.

**Keywords** Phagocytosis, killing, *Candida albicans*, fluoroquinolones, CD11b

**Introduction**

*Candida* species are regularly reported to be the fourth most common cause of bloodstream infection. The incidence of systemic candidosis continues to increase, largely because of the enlarging at risk population [1–4]. Risk factors for *Candida* bloodstream infections can be divided into host-related factors, such as immunocompromising conditions, and healthcare-related factors, such as intravascular catheters, use of broad spectrum antibiotic, and surgical procedures [5,6]. *Candida* infection is a well known independent risk factor of mortality in bloodstream and other infections [7]. In addition, the hospitalization of patients who have had *Candida* infections is more prolonged and expensive compared to those who haven’t had such infections [8].

The role of PMN as the first line of defence against fungi was demonstrated by the observation that patients with prolonged and deep neutropenia and with defective PMN, such as patients suffering from chronic granulomatous disease, are at high risk for fungal infections [9,10]. For phagocytosis by PMN, CD11b expression has been shown to play an important role in the binding and clearance of *Candida albicans* [11].

Most fungal infections show non-specific symptoms, making it difficult to reach the correct diagnoses and causing the use of empirical antiinfective treatment, including antibacterial agents [4]. The impact of antibacterial therapy on invasive fungal infections is complex. First, the increased incidence of fungal infections parallels the decrease in mortality due to bacterial infections. This is probably a result of better antibiotic therapy, thereby leading to increased survival of patients predisposed to fungal infections. Second,
the disruption of the normal microbial flora on the skin and mucosal surfaces caused by in the use of antibiotics is known to be a major risk factor for opportunistic fungal infections [4,6]. Third, antibiotics have been reported to directly influence antifungal defence mechanisms, such as phagocytosis and killing by phagocytes.

As fluoroquinolones (FQ) are commonly used in the empirical treatment of infections which may involve concomitant Candida infections, we focussed on the influence of FQ on phagocytosis and killing of C. albicans by human PMN.

Prior investigations of different FQ showed that these antibacterial agents exert heterogeneous effects on phagocytosis, the burst production and the killing function of the respective phagocytes [12]. While some studies have shown an influence of FQ on phagocyte function [13–17], other investigators did not find any effects of FQ on PMN [18–21]. In light of these conflicting reports we examined this issue by using a systematic approach and different standardized methods. We compared the influence of modern FQ and 6-desfluoroquinolones, as well as older FQ on the activation of human PMN and their phagocytosis and killing of C. albicans.

Materials and methods

Strains and culture conditions

*Candida albicans* wild-type strain ATCC 10261 (human clinical isolate) was used throughout the study. This strain was previously employed in studies of phagocytosis by human neutrophils [22]. For long-term storage, this strain was maintained in a deep freezer (−70°C; Kryobank, MAST Diagnostica, Rheinfeld, Germany).

After thawing, the strain was plated on Sabouraud Dextrose agar (SDA; Merck, Darmstadt, Germany) containing 2% (w/v) glucose and incubated at 30°C for 1 day. One colony from these cultures was suspended in 7 ml culture broth (HHD) and incubated under the same condition until an optical density of 0.6 (590 nm) was reached. Afterwards the culture broth was portioned into 1.5 ml Eppendorf vials (1 ml in each). Yeast cells were harvested by centrifugation and washed with phosphate buffered saline (PBS) as previously described [23]. After resuspension in 1 ml PBS each vial was sonicated (Bandelin Sonorex RK 255H; 320 W, Berlin, Germany) twice for 90 seconds in order to separate the yeast cells from each other.

Susceptibility of *C. albicans* against FQ was tested using a microdilution-technique according to DIN 58940-84 [24] with RPMI 1640 medium (Invitrogen, Karlsruhe, Germany) and FQ up to 2048 mg/l.

Healthy volunteers

Sixty-three healthy volunteers (21 male and 41 female, aged 21–63 years) were enrolled in this study. Volunteers with current infections, immune disorders or severe diseases were excluded. Volunteers were also not allowed to take any of the following substances 14 days prior to their blood donations: antifungal, glucocorticoids, non-steroidal anti-inflammatory substances (except for a single dose more than 3 days before blood donation), anti-histamines, vaccinations, and blood products. The total leukocyte count was determined for each blood donation and if below 4,000/µl or above 11,000/µl, the volunteer was excluded.

The Ethic-Commission of the ‘Ärztekammer Nordrhein’ accepted the study-plan and the information leaflet distributed to the participants.

Fluoroquinolones

The fluoroquinolones (FQ) tested included members of all quinolone-groups that were previously or currently on the market. The study included old non-fluorinated substances like pipemidic acid and cinoxacin, as well as first generation FQ like norfloxacin, lomefloxacin and enoxacin. In addition, current standard FQ such as ciprofloxacin, ofloxacin, levofloxacin, the veterinary FQ enrofloxacin, substances with enhanced spectrum of activity like moxifloxacin, gatifloxacin and sparfloxacin and the 6-desfluoroquinolone garenoxacin were evaluated. The FQ cinoxacin, enoxacin, lomefloxacin, norfloxacin, ofloxacin, pipemidic acid (all from Sigma), ciprofloxacin (AppliChem), moxifloxacin (Bayer Vital, Germany), gatifloxacin (Grünenthal, Germany), levofloxacin (Fluka), sparflaxin and enrofloxacin (both obtained from Axxora, Germany) were dissolved in alkaline aqueous solution, while garenoxacin (Scher-Ing-Plough, USA) was dissolved in dimethyl sulfoxide (DMSO). One to 17.65 µl of appropriate FQ-dilutions were added to 100 µl heparinized whole blood immediately after sampling to reach final concentrations of 0.5, 5, 100, and 1500 mg/l. Identical volumes of PBS or DMSO (garenoxacin) were used as controls. FQ were incubated in the heparinized blood for 15 min prior to the addition of the stained *Candida* cells.

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Phagocytosis

To assess phagocytosis, sonicated yeast cells were incubated with bis-carboxyethyl-carboxyfluoresein-pentaacetoxy-methylester (BCECF/AM, Molecular Probes; final concentration 1 μmol/l) for 30 min at 30°C in 1 ml PBS as described previously [11,22,23]. Non-fluorescent BCECF/AM diffuses into the yeast cells, where it is cleaved by cytoplasmic esterases to yield the fluorescent, membrane impermeable BCECF that remains trapped in viable cells. To achieve a yeast/granulocyte ratio of 2.5:1, the number of yeast cells per volume PBS was determined after staining using a Multisizer TM3 (Coulter). The labelled yeast cells were incubated at 37°C in a thermomixer (Eppendorf, Hamburg, Germany) for a maximum of 60 min in 100 μl heparinized (10 I.E. ≈ ca. 5 USP/ml) whole blood from healthy volunteers at 1000 rpm with and without the respective FQ. At times 0, 5, 15, 30, and 60 min, phagocytosis was stopped by placing the tubes on ice. Fifty μl of the heparinized blood containing yeast cells were stained with anti-CD11b mAbs (please see the section below on CD11b expression) and transferred into 1 ml lysis buffer (Becton Dickinson, Heidelberg, Germany) to lyse the erythrocytes. Within at least 20 minutes, leukocytes and yeast cells were isolated by centrifugation (5 min, 300 g). After washing twice in PBS, cells were resuspended in 500 μl PBS and analysed by flow cytometry.

CD11b expression

CD11b is the beta-chain of the Mac-1 molecule (CD11b/CD18) and has been known for decades to be a major receptor for C. albicans on granulocytes [25]. Antibodies directed to CD11b have been shown to block binding and subsequent phagocytosis of C. albicans by human PMN [11]. An increase of CD11b expression on granulocytes is a manifestation of the activation of these cells [11]. CD11b expression was determined with and without FQ and in the presence and absence of yeast cells so as to determine the direct influence of the FQ on the CD11b expression and the influence of phagocytosis on the CD11b expression. In order to determine CD11b expression, the respective samples were stained prior to the addition of the lysis buffer. Fifty μl of the cold heparinized blood was added to 6.5 μl phycoerythrin-stained anti-CD11b mAbs (Clone D12, Becton Dickinson, Heidelberg, Germany) and incubated on ice for an additional 30 min. Staining of granulocytes with this mAb leads to orange-red fluorescence upon excitation in the flow cytometer and enables simultaneous measuring of CD11b expression and phagocytosis of the green-fluorescent yeast cells [11,23].

Oxidative burst

As the results of the respiratory bursts have previously been shown to parallel the results of the phagocytosis assay, respiratory bursts were indirectly determined. To determine the production of reactive oxygen intermediates (ROI) such as O₂⁻ or OH⁻ and H₂O₂ during the respiratory burst, unlabeled yeast cells were incubated in heparinized blood under identical conditions as described above, except that dihydorhodamine (DHR; Molecular Probes, Eugene, Oreg., USA) was included in the final mixture. DHR was employed at a final concentration of 10 mg/l as described previously [23]. DHR is freely permeable, localizes in the mitochondria of neutrophils and, after oxidation by H₂O₂ and O₂⁻ to rhodamine 123 during the respiratory burst, emits a bright green fluorescent signal upon excitation by blue light (488 nm) [26]. Samples were prepared and analysed by flow cytometry as described above for the phagocytosis assay. Oxidative bursts of the neutrophils induced by unstained yeast cells in the presence of DHR were expressed accordingly as an increase of the green fluorescence of the neutrophils. Blood samples without addition of fungal cells were examined similarly at the beginning and after 60 min as controls.

Flow cytometry

Flow cytometric analysis was performed with a FACS-Calibur flow cytometer (Becton Dickinson) using the Cellquest software for evaluation of data (Becton Dickinson). The instrument’s settings (linear parameters for FSC and SSC, logarithmic parameters for FL1 and FL2) that provided best test performance were very similar to those used previously in experiments with Exophiala dermatitidis and its melanin-deficient mutants [23,27]. Binding of the yeast cells by the neutrophils was analysed as described earlier in the literature [23,27]. Briefly, neutrophils were selectively analysed by gating them according to their relative size and granularity. The association of neutrophils with the BCECF-labelled yeast cells was expressed as an increase of the green fluorescence of the neutrophils. The percentage of neutrophils exhibiting a green fluorescence was determined using the quadrant statistics in the Cellquest-Software (Becton Dickinson). The fluorescence cut-off was set before addition of yeast cells by gating 99% of the neutrophils in a non-fluorescent quadrant.
Killing assay

To quantify the killing of the test strain by human neutrophils, culturing was performed as described above. The harvested and washed yeast cells were diluted in PBS to achieve a ratio of yeast cells and granulocytes of 2.5:1 when adding 50 μl of yeast cells in PBS to 500 μl heparinized blood. Heparinized blood with yeast cells was rotated vertically at 37 °C for 3 h at 6 rpm (Intelli-Mixer RM2, Elmi-Tech). Initially and after 3 h of rotation, 60 μl were removed and diluted with 540 μl of NaCl (0.9% v/v). Viable counts were determined by plating serial dilutions (10⁻² to 10⁻⁴) of all samples onto SDA.

Mean values of these determined counts were calculated and expressed as reciprocal percentage of the respective inoculum indicating the fraction of killed yeast cells.

Statistics

All data were expressed as mean ± SD. Statistical analysis was performed by the non-parametric Wilcoxon test for paired samples. Probability values ≤ 0.05 were considered to be statistically significant.

Results

In light of conflicting reports regarding positive and negative influences of different FQ on the phagocytosis of human PMN, we systematically applied different methods to study the influence of modern fluoro- and 6-desfluoroquinolones, as well as older ones on the phagocytosis and killing of *C. albicans*.

Influence of FQ on Candida albicans

Minimal inhibitory concentrations (MIC) of all FQ on *C. albicans* were determined using standard micro-dilution techniques. Growth was not inhibited by concentrations up to 2,048 mg/l of each tested FQ and thereby excluding a direct inhibitory effect of concentrations ≤ 1500 mg/l.

Influence of FQ on the viability of human PMN

To obtain information on the influence of FQ on the viability of human PMN, we employed the trypan blue staining technique. Human PMN incubated with the tested FQ in concentrations up to 1,500 mg/l for 2 h did not show any significant staining with trypan blue as was the case for the untreated control (data not shown). Therefore, at least membrane integrity as a measure of viability was not disturbed with any of the FQ tested.

Influence of FQ on CD11b expression on human PMN

As shown in Fig. 1a–c, three groups of quinolones could be identified relative to their influence on CD11b expression on non-activated human PMN at high concentrations (1,500 mg/l). In the first group (Fig. 1a), high concentrations (1,500 mg/l) of sparfloxacin, garenoxacin, moxifloxacin and ciprofloxacin increased CD11b expression significantly (5–13 fold, respectively; *P* < 0.05). However, only garenoxacin...
also increased CD11b expression significantly (2fold, \( P<0.05 \)) at clinically relevant concentrations (0.5 and 5 mg/l).

A second group of quinolones (Fig. 1b) including lomefloxacin, ofloxacin, pipemidic acid, cinoxacin and gatifloxacin showed a trend (not significant) to decrease CD11b expression on PMN at high concentrations (please be aware of different scaling of y-axis compared to Fig. 1a).

A third group of quinolones (Fig. 1c) including levofloxacin, enoxacin, enrofloxacin and norfloxacin did not show any influence on CD11b expression on PMN at high concentrations (please be aware of different scaling of y-axis compared to Fig. 1a).

Influence of FQ on the phagocytosis of Candida albicans by human PMN

Among all quinolones tested only ciprofloxacin showed a significant effect on phagocytosis of \( C. \) albicans by human PMN at clinically relevant concentrations (Fig. 2a–d). At 0.5 and 5 mg/l, ciprofloxacin decreased phagocytosis of \( C. \) albicans by human PMN in a concentration dependent manner, to a small (−2.5 to −8.5%, respectively) but significant (\( P<0.03 \)) extent during the first 5 min (Fig. 2a). However, no such effect on phagocytosis could be observed after 15, 30 or 60 min of exposure. With sparfloxacin (Fig. 2d), levofloxacin (data not shown), ofloxacin (data not shown), gatifloxacin (data not shown) a slight decrease in phagocytosis was also observed during the first 5 minutes but only with 100 and 1,500 mg/l (\( P<0.06 \) each). In contrast, ciprofloxacin (Fig. 2a), garenoxacin (Fig. 2b), moxifloxacin (Fig. 2c), and enoxacin (data not shown) significantly inhibited the phagocytosis of \( C. \) albicans by human PMN at high concentrations of 1,500 mg/l. Inhibition was most pronounced with ciprofloxacin (−90%) followed by garenoxacin (−50%), enoxacin (−40%, data not shown) and moxifloxacin (−30%). With enrofloxacin and cinoxacin a decrease in phagocytosis (−45%) was only observed at 1,500 mg/l and then only during the first 5 min (data not shown).

Influence of FQ on oxidative burst after phagocytosis of Candida albicans by human PMN

In the absence of \( C. \) albicans, all FQ tested had no influence on oxidative bursts up to concentrations of 1,500 mg/l (data not shown). As oxidative bursts usually parallel phagocytosis only exemplarily measurements of them have been performed in the presence of \( C. \) albicans. As with phagocytosis a negative effect on oxidative bursts by −80, −60 and −30% was only shown with high concentrations of ciprofloxacin, garenoxacin and moxifloxacin, respectively (Fig. 3).
Influence of FQ on the killing of Candida albicans by human PMN

In order to determine the net effects of FQ on the phagocytic function of human PMN we assessed the influence on killing of Candida albicans during the incubation period of 4h in whole blood. As shown in Fig. 4 garenoxacin, moxifloxacin and ciprofloxacin did not hamper the killing of C. albicans by human PMN at clinically relevant concentrations. This was also shown for other tested quinolones (data not shown), e.g., ofloxacin, levofloxacin, gatifloxacin, enrofloxacin, enoxacin, cinoxacin, lomefloxacin, pipemidic acid. However, killing was significantly increased by high concentrations of norfloxacin and sparfloxacin. However, killing of non-proliferating C. albicans in phosphate buffered saline (PBS) was not affected by these high concentrations confirming the results showing no direct growth inhibitory effect of high concentrations up to 1,500 mg/l.

Discussion

Candida albicans is a common opportunistic fungal pathogen and the leading cause of invasive fungal disease. Protective immunity to disseminated Candida infections has traditionally been ascribed to innate immunity with emphasis on the role of PMN. Although immunological studies have revealed that host defence against Candida infections is based on a complex interplay between innate and cell-mediated immunity, the important role of PMN is generally not questioned [28,29]. Within PMN, both myeloperoxidase and NADPH-oxidase have been shown to critically contribute to early host defence against Candida albicans [30]. The importance of PMN was recently strengthened by the observation that C. albicans is able to specifically shed Pra1p, the soluble form of the fungal protein responsible for the binding of C. albicans to CD11b/CD18 of neutrophils, thereby evading the host innate defence [31].

Due to the non-specific clinical presentation of fungal infections and the frequent concomitant infections with bacteria, the administration of FQ during Candida infections is not unusual. The influence of FQ on phagocytes has been and still is controversial in literature [12]. Whereas some studies indicated an inhibition of phagocytosis or the oxidative burst by several FQ [13–16], others revealed an increased oxidative burst by some FQ [15,16] or even an increased killing [17]. On the other hand, several studies did not reveal any significant influence of FQ on phagocyte functions [18–21]. These differences in the findings of reports in the literature might be – at least in part – due to the use of different methods. In most of the studies, isolated human PMN [13,16,17,20,21] and in one study porcine PMN [19] were employed. Isolation of PMN is known to cause artificial changes such as altered expression of surface antigens [32], morphologic changes [33], aggregation and activation of PMN [34] and reduction of the oxidative burst, chemotaxis and increased release of lysosomal enzymes [35]. The use of whole blood for investigation has been shown to markedly reduce the activation of PMN prior to phagocytosis assays [36,37].
In this study, we used 10 IE of heparin per ml of blood for anticoagulation. This concentration of heparin has previously been shown to reduce anticoagulation-immanent activating effects on PMN [36–38].

Four of the FQ-studies used living microorganisms enabling measurement of phagocytosis and killing [17–20]. Two of them were conducted using *C. albicans* [18,20], with isolated human PMN [20] in one and heparinized whole blood in the other [18]. In each of these two investigations only one FQ (moxifloxacin [18] or lomefloxacin [20]) was studied, and both found no significant influence of these FQ on the killing, oxidative burst and phagocytosis of *C. albicans* by human PMN.

In the present study, we used native vital stained instead of killed *Candida albicans* cells to exclude effects of the killing-procedure on the surface of the *Candida* cells. We did not pre-opsonize the cells, because incubation time within whole blood during the tests ensured sufficient opsonization of the target cells for phagocytosis in a physiologic environment [39,40].

For the same reasons we used the intracellular dye BCECF-AM to stain the yeasts. This method was described previously [11,23,41]. The colourless ester BCECF-AM passes the lipophile membrane of *Candida* cells. The intracellular cleaving of the ester leads to the product BCECF generating a green fluorescence upon excitation. BCECF remains trapped in viable cells and has been shown to exhibit no toxic effects on the target cells and PMN [11,23,36,37,41,42].

For the determination of the oxidative burst activity a similar principle is used in nearly all previously described assays. During the oxidative burst the colourless dye dihydrorhodamine (DHR) is oxidized and thereafter becomes detectable by flow cytometry. DHR is a sensitive intracellular dye not influencing the characteristics of the PMN and, after oxidation, emits a green fluorescence upon excitation [26,43]. As its fluorescence interferes with the fluorescence of BCECF-stained *Candida* cells in the phagocytosis assays, oxidative burst was studied with unstained *Candida* cells in simultaneously conducted assays. As previously shown, oxidative bursts often parallel the results of phagocytosis using yeast cells [11,18,22,23]. Therefore, oxidative burst was only determined exemplarily. This approach was justified by almost identical results for phagocytosis and oxidative bursts.

Compared to previous studies we improved the standardization of the assays by automatic cell counts for both PMN and yeast cells, to reveal a stable ratio of the microorganisms to the PMN which has been known to be a critical parameter for reproducibility [17]. Additionally, the study protocol contributed to a further standardization as several conditions which might affect the PMN function were excluded.

None of the previous studies analysed the influence of FQ on the CD11b expression of PMN. As Mac-1 (CD11b/CD18) is a major receptor for *C. albicans* [11,25], modulation of Mac-1 expression most probably would influence phagocytosis of *C. albicans*. The current study showed that none of the FQ tested exhibited any clinical significant influence on the CD11b expression at therapeutic concentrations. However, garenoxacin, a new des-fluorinated quinolone, increased basal CD11b expression at therapeutic relevant concentrations by a factor of 2–2.8 compared to control. While this change was statistically significant, no clinical impact is expected because normal activation of PMN (e.g., with opsonized microorganisms) increased CD11b expression rapidly, up to >10 times above basal level [11]. Correspondingly, no significant influence of garenoxacin on CD11b expression has been shown in the presence of *C. albicans* at any concentration tested (data not shown). As CD11b expression was mostly unaffected by tested FQ, the same applies for phagocytosis of *C. albicans*. Only ciprofloxacin exhibited a small but statistically significant delay of phagocytosis (−8.5%) during the first 5 min of exposure. However, this small and temporary impairment of phagocytosis has probably no clinical impact on the defence of microorganisms by PMN.

None of the studies conducted thus far allowed for comparison of all FQ classes. As some studies indicated a great impact of the FQ concentration on the results [14–16], we tested a broad range of FQ concentrations up to 1,500 mg/l. Therefore, for the first time, we are able to compare the influence of the most important FQ on the phagocytosis, the oxidative burst, the CD11b expression and the killing of *C. albicans* by PMN in a concentration dependent manner, excluding artificial effects of PMN isolation and surface alteration of the microorganisms.

Limitations of our methods are the *in vitro* conditions in our assays and the short incubation of the FQ on the PMN. As PMN get activated during *in vitro* incubation [44], all *in vitro* assays have to compromise between incubation period and PMN activation. We tested several agitation methods during incubation and their influence on PMN activation. Dependent on the results, we choose the method with the lowest potency to activate the PMN during the needed incubation time (data not shown). The influence of this methodological activation on the results was minimized by respective controls. The flow cytometric phagocytosis assay is not
able to differentiate between adhered and ingested yeast cells. Previous studies using epifluorescence interference contrast microscopy to control the ingestion of yeast cells under the conditions used, clearly showed that the yeast cells bound to the PMN are immediately ingested by the PMN [11,23]. Additionally, the results of the killing assays support the ingestion of the yeast cells by the PMN as this is essential for effective killing.

Thus, we can conclude that none of the tested FQ at therapeutic relevant concentrations exhibit a clinically relevant influence on the phagocytosis, oxidative burst and killing of C. albicans by human PMN. These results confirm previous investigations by Fischer, who also used heparinized whole blood showing no such influences of moxifloxacin up to 100 mg/l [18].

In contrast, while testing high non-therapeutic concentrations (1,500 mg/l) we could demonstrate that FQ do not behave equally in influencing CD11b expression, phagocytosis, oxidative burst and killing. Whereas some FQ increased basal CD11b expression in the absence of microorganisms by factors of 5 (sparfloxacin), 6 (garenoxacin), 8 (moxifloxacin) or even 13 (ciprofloxacin), others seemed to have no effect (levofloxacin, enoxacin, enrofloxacin, norfloxacin) or even tend to decrease basal CD11b expression by maximally 50% (lomefloxacin, ofloxacin, pipemidic acid, cinoxacin or gatifloxacin). It is noteworthy that only those FQ which increased basal CD11b expression in the absence of microorganisms, exhibited a negative effect on phagocytosis and oxidative burst at 1,500 mg/l, with exception of enoxacin. These FQ, i.e., ciprofloxacin, sparfloxacin, moxifloxacin, garenoxacin and norfloxacin or even exhibit a cyclopropyl-moiety at the position N1 of the quinolone core structure. However, enrofloxacin which also exhibits this cyclopropyl-moiety did not increase CD11b expression nor diminish phagocytosis or oxidative burst at 1,500 mg/l. A recent review of immunomodulatory effects of fluoroquinolones, especially influences on cytokine production concludes that these effects can be attributed in particular to those FQ with a cyclopropyl-moiety at the position N1 of the quinolone core structure, i.e., ciprofloxacin, moxifloxacin, grepafloxacin, and sparfloxacin [45].

One possible explanation for this phenomenon might be a pronounced direct toxic effect of these FQ at 1,500 mg/l on the PMN leading to impairment of DNA metabolism and apoptosis. Former experiments have shown that apoptotic neutrophils upregulated CD11b and CD11c by about 50%, whereas other integrins (e.g., CD11a) were down regulated [46]. However, trypan blue staining of PMN did not reveal any significant impairment of viability. Alternatively, fluoroquinolones may also exhibit negative effects on Mac-1 receptor affinity or avidity resulting in hampered phagocytosis and oxidative burst. Increased affinity and avidity of MAC-1 is necessary for optimal phagocytic action of PMN [47–49]. A key element in activation of PMN and subsequent phagocytosis is intracellular calcium flux [49]. Chelation of Ca$^{2+}$ by EGTA has been shown to repress activation of PMN by zymosan, an insoluble carbohydrate from the cell wall of yeast [50], and to partly inhibit the phagocytosis of Saccharomyces cerevisiae by neutrophils [51]. As FQ are potent chelators of divalent cations [52], chelation of Ca$^{2+}$ and Mg$^{2+}$ might also contribute to the observed effects on phagocytosis and oxidative burst of C. albicans at high FQ concentrations. However, as chelation of Ca$^{2+}$ and Mg$^{2+}$ with EDTA has also been shown to decrease CD11b expression on human PMN [53], the observed increase in CD11b expression by ciprofloxacin, sparfloxacin, moxifloxacin and garenoxacin cannot be explained with chelation of Ca$^{2+}$ and Mg$^{2+}$.

The observed increased killing of C. albicans with 1,500 mg/l of norfloxacin and sparfloxacin is unexplained. Both FQ might exhibit either a more pronounced direct toxic effect on C. albicans (although MIC was $>$ 2,048 mg/l) or on PMN, as stimulation of PMN apoptosis and release of antimicrobial substances might contribute to an increased killing of C. albicans.

Our results show significant effects, with probable clinical impact, of the FQ only at very high concentrations. However, these effects resemble structural differences within tested FQ. This might, at least partly, explain some of the differences observed in previous studies.

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