Activity of moxifloxacin against *Bacteroides fragilis* and *Escherichia coli* in an *in vitro* pharmacokinetic/pharmacodynamic model employing pure and mixed cultures

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The objective of this study was to determine the pharmacodynamic (PD) activity of moxifloxacin against four selected *Bacteroides fragilis* strains (three strains with low MICs and one strain with a high MIC) and two *Escherichia coli* strains (one strain with a low MIC and one strain with a high MIC) in a pharmacokinetic (PK) *in vitro* model in pure cultures as well as in mixed cultures. PK/PD assays of moxifloxacin were carried out with an initial maximum concentration of 4·0 mg l^{-1} and a half-life of 13 h. The *E. coli* strain with the low MIC was rapidly killed in both pure and mixed cultures in the *in vitro* PK/PD model, while the *E. coli* strain with the high MIC was not killed. None of the *B. fragilis* strains were rapidly killed in pure or mixed cultures. The bacterial numbers of the *B. fragilis* strains with low MICs were reduced by about one to two logs after 12 h in pure cultures. The presence of an *E. coli* strain with a low or a high MIC in the mixed culture reduced this effect even further.

INTRODUCTION

Obligately anaerobic bacteria comprise the largest part of the physiological flora on skin and mucous membranes of humans. Since they are opportunistic pathogens, they often participate in endogenous infections, causing mixed infections together with aerobic bacteria. Such infections (i.e. intra-abdominal) are burdened with high morbidity and mortality, and require treatment with antimicrobial drugs showing activity against both aerobic and anaerobic bacteria (Gorbach, 1994; Nathens & Rotstein, 1996; Olsen *et al.*, 1999).

Moxifloxacin is a quinolone that, like trovafloxacin and clinafloxacin, belongs to the fluoroquinolone group IV as defined by Naber & Adam (1998). It has an antimicrobial activity against many Gram-positive and Gram-negative aerobic and anaerobic bacteria as well as atypical bacteria such as *Chlamydia* and *Mycoplasma* (Dalhoff *et al.*, 1996; Bauernfeind, 1997; Goldstein *et al.*, 1997; Edlund *et al.*, 1998; Ackermann *et al.*, 2000; Blondeau *et al.*, 2000; Schaumann *et al.*, 2000; Kleinkauf *et al.*, 2001; Krasemann *et al.*, 2001; Talan, 2001; Zhanel *et al.*, 2002).

Several *in vitro* studies have indicated that moxifloxacin has good *in vitro* activity against important anaerobic bacteria especially *Bacteroides* species (Edlund *et al.*, 1998; Ackermann *et al.*, 2000). However, compared to the activities of garenoxacin, clinafloxacin, sitafloxacin and trovafloxacin, moxifloxacin more recently was the least active agent against 589 *Bacteroides fragilis* group isolates (Snydman *et al.*, 2002). Furthermore, fluoroquinolone resistance among *Bacteroides* isolated in the USA has apparently markedly increased since 1994 (Golan *et al.*, 2003). Hedberg & Nord (2003) reported that antimicrobial resistance among *B. fragilis* group isolates in Europe is also increasing. Conversely, cidal moxifloxacin activity was found for respiratory pathogens (aerobes and anaerobes) even when sera were obtained 24 h after dosing. The results suggest that moxifloxacin may have clinical utility in the treatment of mixed aerobic/anaerobic respiratory tract infections (Stein *et al.*, 2003b). In a recently published paper investigating serum bactericidal activity of moxifloxacin and gatifloxacin, Stein *et al.* (2003a) reported little or no serum bactericidal activity of either drug if the MICs of gatifloxacin were ≥2 mg l^{-1}. However, moxifloxacin was found to be effective *in vivo* even against a *B. fragilis* strain with a high MIC level for moxifloxacin in an experimental animal model of severe mixed aerobic/anaerobic infection (Schaumann *et al.*, 2004).

The aim of the present study was to assess the killing activity of moxifloxacin in an *in vitro* pharmacokinetic/pharmacodynamic (PK/PD) model against four selected *B. fragilis* strains used previously in the *in vivo* experimental model (Schaumann *et al.*, 2004). Since anaerobes are often present in mixed infections, kill kinetics were also established for mixed inocula employing the *B. fragilis* strains together with one of two different *Escherichia coli* strains, one strain with a low MIC for moxifloxacin and one with a high MIC.

METHODS

Bacterial strains. *E. coli* ATCC 25922, *E. coli* VA 6886 and different strains of *B. fragilis* (RMA 0309, RMA 5120, RMA 6791, WAL R 13267) were used. *E. coli* VA 6886 was isolated from bile at the Institute of Medical Microbiology of Leipzig, Germany. RMA 0309 and RMA 5120 were intra-abdominal isolates, RMA 6791 was a blood culture isolate and WAL R 13267 was a clinical isolate of unknown origin.

The *B. fragilis* strains and *E. coli* ATCC 25922 strain were characterized as follows as described previously (Schaumann *et al.*, 2004): *B. fragilis* RMA 0309, enterotoxin negative, MIC for moxifloxacin 0·125 mg l^{-1}; *B. fragilis* RMA 5120, enterotoxin negative, MIC for moxifloxacin 0·125–0·38 mg l^{-1}; *B. fragilis* RMA 6791, enterotoxin positive, MIC for moxifloxacin 0·25–0·5 mg l^{-1}; *B. fragilis* WAL R 13267, enterotoxin positive, MIC for moxifloxacin >32 mg l^{-1}; *E. coli* ATCC 25922, MIC value for moxifloxacin <0·03 mg l^{-1}.

The MIC for the *E. coli* strain VA 6886 was established by broth microdilution technique according to DIN 58940-8 (Deutsches Institut für Normung e.V., 2000). The result was confirmed by E-test (AB BIODISK) according to the manufacturer's instructions, resulting in a MIC value of >32 mg l^{-1}.

The *E. coli* strains were grown on Endo agar (bioMérieux) and the *B. fragilis* strains were grown on Columbia agar (Oxoid) supplemented with 5 % sheep blood (Oxoid), vitamin K1 (Sigma) and haemin (Serva Feinbiochemica). After incubation, *B. fragilis* strains and *E. coli* strains were harvested from the plates and suspended separately in Brucella broth (Becton Dickinson) supplemented with vitamin K1 and haemin, and incubated overnight at 37 °C under anaerobic and aerobic conditions, respectively. Then the suspensions were adjusted turbidimetrically to contain approximately 1·2 × 10^9 c.f.u. ml^{-1} *B. fragilis* and approximately 1·5 × 10^8 c.f.u. ml^{-1} *E. coli*. Cultures for the experimental model were set up as described below and the numbers of bacteria were confirmed by appropriate plating.

Antimicrobial agent. Moxifloxacin powder of known activity was kindly provided by Bayer Vital and suspended in distilled water.

Experimental model. In order to determine the pharmacodynamic activity of moxifloxacin all six strains were investigated in pure cultures as well as in mixed cultures. Cultures were set up in a final volume of 20 ml of appropriately supplemented Brucella broth with approximately 2·4 × 10^8 c.f.u. ml^{-1} *B. fragilis* or approximately 3 × 10^7 c.f.u. ml^{-1} *E. coli* or both, and an initial maximum concentration (C_{max}) of 4·0 mg l^{-1} moxifloxacin. The *in vitro* pharmacokinetic assays for moxifloxacin were carried out over 12 h with $t_{1/2}$ of 13 h according to the equation $C_t = C_0 \times e^{-k_{el} \times t}$ (C_t = concentration of moxifloxacin at a given point in time (t); C_0 = initial concentration of moxifloxacin). The elimination rate constant (k_{el}) was calculated using the equation $k_{el} = \ln 2/t_{1/2}$.

An observation time period of 12 h was chosen according to the half-life of moxifloxacin and the dosing interval suggested for intra-abdominal infections. We used a C_{max} concentration for moxifloxacin of 4 mg l^{-1}

equal to total concentration since protein binding of moxifloxacin probably occurs in culture media as well. High inocula of approximately 2·4 × 10^8 c.f.u. ml^{-1} *B. fragilis* and approximately 3 × 10^7 c.f.u. ml^{-1} *E. coli* were used since abscesses contain a large number of bacteria (Stearne *et al.*, 2001 & 2002).

The PK/PD model was established by adding appropriate amounts of supplemented Brucella broth every 30 min (540–980 µl), resulting in a final volume of 36·25 ml and a final moxifloxacin concentration of 2·11 mg l^{-1} after 12 h. At 30 min intervals samples (20 µl) were taken and diluted aliquots were plated on Endo agar as well as on supplemented Columbia agar. The experiments were carried out in an anaerobic chamber (Heraeus) containing 80 % N$_2$, 15 % CO$_2$ and 5 % H$_2$ at 37 °C. However, the Endo agar plates were incubated under aerobic conditions for 24 h. After incubation, bacterial colonies were counted and calculated to colony forming units per ml. The detection limit was 1 × 10^2 c.f.u. ml^{-1}. In mixed cultures it was macroscopically possible to distinguish the colonies of *E. coli* from the colonies of *B. fragilis* strains growing on Columbia agar due to morphological criteria.

Statistical analysis. Mann trend test was used for testing for trends in a time-series of killing ratios (Hartung, 1999; Hollander & Wolfe, 1999). A P value of <0·05 was considered to be significant.

RESULTS AND DISCUSSION

For our PK/PD assay we employed a reasonably high initial concentration of moxifloxacin (4 mg l^{-1}) that was reduced to approximately 2·11 mg l^{-1} after 12 h. In pure cultures the bacterial numbers of *B. fragilis* strains with low MICs (≤0·5 mg l^{-1}) were moderately but significantly reduced (by about one to two logs) by moxifloxacin within 12 h (killing rates: RMA 0309, 99·3 %; RMA 5120, 99·2 %; RMA 6791, 99·2 %; P < 0·01). For *B. fragilis* WAL R 13267 (MIC >32 mg l^{-1}) no reduction was observed (P > 0·05). Thus, none of the *B. fragilis* strains was effectively killed in pure culture (Fig. 1).

The *E. coli* ATCC 25922 strain (MIC < 0·03 mg l^{-1}) was rapidly killed by moxifloxacin both in pure (data not shown) and mixed cultures (Fig. 2a), confirming a bactericidal effect against this strain (P < 0·01; killing rate within the first hour >99·9 %). As was to be expected, there was no bactericidal effect of moxifloxacin against the *E. coli* strain VA 6886 (MIC value: >32 mg l^{-1}) at the moxifloxacin concentrations employed here (Fig. 2b; P > 0·05; data of pure culture not shown).

In mixed cultures employing *E. coli* strain ATCC 25922, with the low moxifloxacin MIC, the bacterial numbers of *B. fragilis* strains with low MICs were significantly reduced (killing rates: RMA 5120, 90·5 %; RMA 6791, 91·8 %; RMA 0309, 99·8 %; P < 0·01; Fig. 2a) and the growth rate of the *B. fragilis* strain with a high MIC (WAL R 13267) was significantly lower compared to the control without moxifloxacin (P < 0·01). Nevertheless, in the presence of the *E. coli* strain ATCC 25922 the moderate activity of moxifloxacin against the anaerobes (except strain RMA 0309) was significantly reduced as compared to single anaerobic cultures (P ≤ 0·01). In mixed cultures containing the *E. coli* strain VA 6886 and the *B. fragilis* strains with low MICs, the bacterial
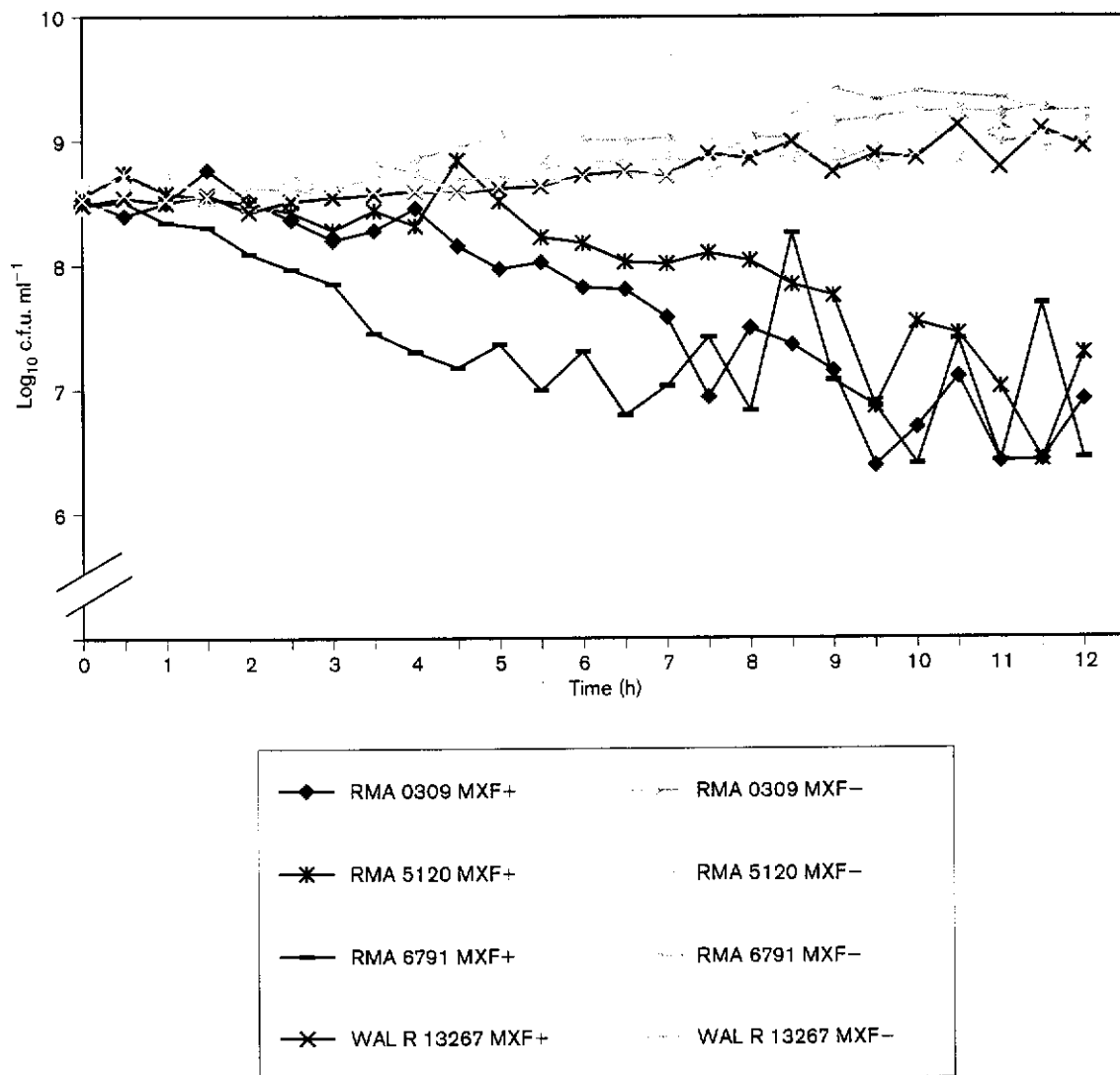
Fig. 1. Activity of moxifloxacin against four different strains of *B. fragilis* tested in the *in vitro* PK/PD model in pure cultures with and without moxifloxacin. MXF+, with moxifloxacin; MXF−, control without moxifloxacin. Detection limit: 10^2 c.f.u. ml^{-1}.

numbers of these bacteria were again significantly reduced compared to the control (killing rates: RMA 0309, 72·2 %; RMA 5120, 82·7 %; RMA 6791, 88·2 %; $P < 0.01$; Fig. 2b) but significantly less reduced if compared to pure cultures with moxifloxacin ($P < 0.01$). Comparing the effects of the two *E. coli* strains on the anaerobes a significant difference in killing was only found for *B. fragilis* RMA 0309 ($P < 0.01$). In addition, the growth of the *B. fragilis* WAL R 13267 strain was significantly lower in the presence of the *E. coli* VA 6886 strain when compared to pure culture or to the mixed culture with *E. coli* ATCC 25922 ($P < 0.01$). However, the growth of the *B. fragilis* WAL R 13267 strain was not reduced by moxifloxacin compared to the initial inoculum.

In contrast to the moderate activity of moxifloxacin investigated here using the *in vitro* PK/PD model, moxifloxacin has been shown to be as efficacious as imipenem/cilastatin in

the treatment of severe systemic mixed aerobic/anaerobic infection in mice using the same *B. fragilis* strains and *E. coli* strain ATCC 25922 (Schaumann *et al.*, 2004). Earlier observations of Onderdonk *et al.* (1976) suggested that *E. coli* is primarily responsible for the lethal effects in the animals. Therefore, the results of the PK/PD model support those previous observations since in the *in vitro* PK/PD model, moxifloxacin was fully bactericidal against the *E. coli* strain ATCC 25922. The presence of the *E. coli* ATCC 25922 and VA 6886 strains reduced the killing activity of moxifloxacin against the *B. fragilis* strains investigated in the *in vitro* PK/PD model. This observation is supported by earlier results obtain with trovafloxacin (Stearne *et al.*, 2001). Furthermore, protection of *B. fragilis* against the activity of metronidazole by *Enterococcus faecalis* has also been observed (Nagy & Földes, 1991). Nagy & Földes (1991) reported that metronidazole was inactivated by a cell extract of *E. faecalis*.

antimicrobial agent. However, further investigations would be required to confirm this hypothesis. Because of the results reported here, the clinical study data obtained for moxifloxacin treatment of patients with intra-abdominal infections need intensive scrutiny.

ACKNOWLEDGEMENTS

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