Effect of moxifloxacin versus imipenem/cilastatin treatment on the mortality of mice infected intravenously with different strains of Bacteroides fragilis and Escherichia coli

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Objectives: To study the effect of moxifloxacin versus imipenem (hereafter referred to as imipenem) treatment on the mortality of mice infected intravenously with different strains of Bacteroides fragilis and Escherichia coli.

Methods: Groups of 20 mice each were infected intravenously with different strains of B. fragilis [moxifloxacin and imipenem susceptible or resistant, and enterotoxin (ET) positive or negative] and E. coli (moxifloxacin and imipenem susceptible). Twenty-four hours post-infection, intravenous therapy with either moxifloxacin (2.0 mg twice a day) or imipenem (2.4 mg three times a day) was started and continued for 3 days. Control groups were left untreated. Survival rates were recorded at day 7 post-infection. At that time, surviving mice were killed and numbers of bacteria in the liver and kidneys were determined.

Results: If compared with untreated animals, mice treated with either moxifloxacin or imipenem showed significantly improved survival (P < 0.001). There was no significant difference (P = 0.97) in the survival rates comparing the two treatment regimens irrespective of the ET positivity or the susceptibility to moxifloxacin or imipenem of the infective B. fragilis strain. However, there was a tendency that B. fragilis was recovered more often from the liver and kidneys of mice infected with ET positive strains.

Conclusions: The data show that moxifloxacin was as efficacious as imipenem in reducing the mortality rate of mice suffering from a severe systemic aerobic/anaerobic infection.

Keywords: aerobic/anaerobic infections, mouse model of infection, treatment of mixed infections

Introduction

Mixed aerobic/anaerobic infections such as intra-abdominal sepsis are serious infections with high morbidity and mortality requiring treatment with antimicrobial drugs that are active against both aerobic and anaerobic bacteria.1–3

Moxifloxacin is a new quinolone carboxylic acid derivative with activity against many Gram-positive bacteria, Gram-negative aerobic and anaerobic bacteria and the so-called atypical pathogens such as Chlamydia and Mycoplasma.4–13 It is available as oral and parenteral formulations for the treatment of respiratory tract infections.11,13,14 Several studies have indicated that moxifloxacin has a good in vitro activity against important anaerobic bacteria, especially Bacteroides species.14,15 For example, the results of Ackermann et al.4 indicated that moxifloxacin was almost as active as trovafloxacin, as active as gatifloxacin, and more active than levofloxacin and ciprofloxacin against 292 anaerobes tested. In contrast, more recently, moxifloxacin was the least active quinolone when compared with the activities of garenoxacin, clinafloxicin, sitafloxacin and trovafloxacin against 589 Bacteroides fragilis group isolates, especially Bacteroides thetaiotaomicron.16 Moreover, Golan et al.15 reported that fluoroquinolone resistance among Bacteroides isolated in the USA markedly increased during the years 1994–2001. Investigating the activity of ampicillin, cefoxitin, clindamycin, moxifloxacin, imipenem, piperacillin/tazobactam and metronidazole, Hedberg & Nord17 reported that the antimicrobial resistance among B. fragilis group isolates in Europe is also increasing. Hence, the therapeutic potential of moxifloxacin in mixed aerobic/anaerobic infections is under discussion.18,19

Earlier, Rodloff et al.20–22 described an experimental model of systemic mixed aerobic/anaerobic infection in mice employing intravenous administration of Escherichia coli and B. fragilis. In this...
study, we used this experimental model to assess the therapeutic efficacy of moxifloxacin in mice infected with different strains of B. fragilis (either moxifloxacin susceptible or resistant; breakpoints ≤1/2/4 mg/L) and E. coli (moxifloxacin susceptible). In addition, the B. fragilis strains employed here were characterized as either enterotoxin (ET) positive or negative. According to Kato et al. and Claros et al., ET positive B. fragilis strains are more often isolated from blood culture than any other source. This suggests that ET is a virulence factor in diseases other than diarrhoea and might contribute to invasiveness of the strain.

To compare the results with a standard therapeutic regimen, imipenem/cilastatin (hereafter referred to as imipenem) was chosen. The in vitro and in vivo activity of imipenem against aerobic and anaerobic bacteria is well documented in previous studies. In a recently published study, less than 1% out of 1284 B. fragilis group isolates were resistant to imipenem (breakpoint in this study: 16 mg/L). Moreover, the therapeutic efficacy of imipenem in severe systemic infections in humans is well documented.

Materials and methods

Animals

Specific pathogen free, female (C57B16xDBA2)F1 mice –10 weeks old, weighing around 22 g, were obtained from Harlan-Winkelmann GmbH (Borchen, Germany). The animals were housed at the animal care facility at our institution and food and water were provided ad libitum.

Bacterial strains

E. coli ATCC 25922 and different strains of B. fragilis (RMA 0309, RMA 5120, RMA 6791, WAL R 13267) were used. RMA 0309 and RMA 5120 were intra-abdominal isolates, RMA 6791 was a blood isolate and WAL R 13267 was also a clinical isolate of unknown origin. The strains RMA 0309, RMA 5120 and RMA 6791 were kindly provided by E. J. C. Goldstein, R. M. Alden Research Laboratory, Santa Monica, CA, USA and WAL R 13267 was collected from international anaerobe studies.

The B. fragilis strains were differentiated by PCR for ET gene positivity as described previously by Claros et al. In brief, 10 colonies of each isolate were used for preparation of the DNA with a DNeasy Tissue Kit (Qiagen GmbH, Hilden, Germany). PCR assay for enterotoxigenic E. coli was carried out with the RS-3–RS-4 primer pair (sequences described by Claros et al. and Shetab et al.). The first cycle consisted of 2 min at 95°C, followed by 45 cycles each with 1 min at 95°C, 1 min at 65°C and 1 min at 72°C. After one final cycle of 2.5 min at 72°C, the product samples were obtained and kept at 4°C until further analysis. The amplified products were detected in a 1.5% agarose gel (Amersham Pharmacia Biotech AB, Uppsala, Sweden) stained with 0.5 mg/L ethidium bromide (Sigma Chemical Co., St Louis, MO, USA).

For the experiments, B. fragilis strains were grown on Columbia agar (Oxoid Ltd, Basingstoke, UK) supplemented with 5% sheep blood (Oxoid GmbH, Wesel, Germany), vitamin K1 (Sigma Chemical Co.) and haemin (Serva Feinbiochemica, Heidelberg, Germany). After 48 h of incubation in an anaerobic atmosphere at 37°C B. fragilis strains were harvested from the plates and suspended in brain heart infusion broth (Oxoid). These cultures were incubated anaerobically for 18 h at 37°C. Then the suspensions were adjusted turbidimetrically to 2×10^8 cfu/mL. E. coli was incubated aerobically for 16 h at 37°C on Columbia agar supplemented with 5% sheep blood. Again, strains were harvested from the plates, suspended in glucose broth (Oxoid) and incubated for 4 h at 37°C. The suspension was also adjusted turbidimetrically to 2.5×10^8 cfu/mL. Colony forming units of B. fragilis and E. coli were confirmed by appropriate plating.

Antibiotics

Moxifloxacin powder of known activity was kindly provided by Bayer Vital GmbH (Leverkusen, Germany) and suspended in Aquadest. Imipenem/cilastatin and physiological saline solution were supplied by MSD Sharp & Dohme GmbH (Haar, Germany). The injected doses were calculated according to the formula of Ungemach. Since moxifloxacin has a considerably shorter half-life in mice than in humans, twice the calculated dose of moxifloxacin was administrated. After an intravenous administration of 9.2 mg/kg moxifloxacin in mice and 1.2 mg/kg in humans, t1/2 was 0.93 h in mice and 13 h in humans, respectively.

MIC values

MIC values for E. coli were established by the broth microdilution technique according to DIN 58940–8,38 and by Etest (AB BIODISK, Solna, Sweden) according to the manufacturer’s instructions. The MIC values for the different B. fragilis strains were also established by Etest according to the manufacturer’s instructions as described previously by Ackermann et al. Briefly, the Etest was carried out on supplemented Columbia agar with a 1 McFarland standard-matched inoculum. Since breakpoints for moxifloxacin for anaerobes have not been determined by the NCCLS or IN, in accordance with other studies, the following breakpoints (mg/L, susceptible/intermediate/resistant) were used: ≤1/2/4.

For imipenem, breakpoints approved by DIN were used: ≤2/4/8.

Experimental model

Two hundred and forty mice were randomly divided into groups of 20 mice each. The mice were infected with RMA 0309 and E. coli (60 mice), RMA 5120 and E. coli (60 mice), RMA 6791 and E. coli (60 mice), or WAL R 13267 and E. coli (60 mice). The inoculum was given by intravenous tail vein injection of 0.2 mL of bacterial suspension containing 2×10^8 B. fragilis and 2.5×10^6 E. coli organisms. Groups of 20 mice each were treated with either moxifloxacin or imipenem, or were left untreated (control groups). Treatment was started 24 h post-infection by intravenous tail vein injection with moxifloxacin (2.0 mg twice a day) or imipenem (2.4 mg imipenem and 2.4 mg cilastatin three times a day) and continued for 3 days. In addition, one group of 20 mice was left without infection and without treatment. The infection with the different B. fragilis strains and E. coli of all 240 mice and the treatment 24 h post-infection, respectively, was started on the same day. Survival was recorded at day 7 post-infection. Then surviving mice were killed and numbers of bacteria in the liver and kidneys were determined.

Bacterial organ contents

The liver and kidneys were homogenized in 10 mL of physiological saline solution and diluted aliquots were plated on Endo agar (bioMérieux, Marcy l’étoile, France) as well as on supplemented Columbia agar. Endo agar plates were incubated aerobically for 24 h at 37°C, and Columbia agar plates anaerobically for 48 h at 37°C. Then colony forming units of E. coli and B. fragilis were determined. The detection limit was 1×10^2 bacteria per homogenate.

Statistics

Statistical analysis was carried out by χ² test comparing the mortality/survival rate. A P value <0.05 was considered to be significant.

Ethical approval

Ethical approval was given by the appropriate regional government authorities, Leipzig, Germany (reference: 74-9168.11-28/00).
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Results

MIC values of moxifloxacin and imipenem for E. coli were <0.03 and 0.125 mg/L, respectively, indicating that E. coli was susceptible to both moxifloxacin and imipenem. MIC values (mg/L) of moxifloxacin and imipenem for the different strains of B. fragilis were 0.125 and >32 (RMA 0309 strain), 0.125–0.38 and 0.064–0.09 (RMA 5120 strain), 0.25–0.5 and 0.5–1.0 (RMA 6791 strain), and >32 and 0.06–0.125 (WAL R 13267 strain). Figure 1 shows the amplified ET genes as visualized with an ethidium bromide-stained agarose gel. RMA 0309 and RMA 5120 strains were negative for the ET genes whereas the RMA 6791 and WAL R 13267 strains were positive. Thus, the characteristics of the four B. fragilis strains used here were as follows:

B. fragilis RMA 0309: ET negative (r)-moxifloxacin susceptible(s);
B. fragilis RMA 5120: ET negative (r)-moxifloxacin susceptible(s);
B. fragilis RMA 6791: ET positive (s)-moxifloxacin susceptible(s);
B. fragilis WAL R 13267: ET positive (s)-moxifloxacin resistant(r), imipenem susceptible(s).

None of the untreated mice died. The mortality rate of the untreated mice was 40% in the group infected with B. fragilis RMA 0309 (ET(r)-moxifloxacin(s)-imipenem(r)) and E. coli, 45% in the group infected with B. fragilis RMA 5120 (ET(r)-moxifloxacin(s)-imipenem(r)) and E. coli, and 50% in the group infected with B. fragilis RMA 6791 (ET(s)-moxifloxacin(s)-imipenem(s)) and E. coli. Within 24 h post-infection, 0–2 mice died in the control and treatment groups before the onset of therapy (Table 1). Overall, if compared with untreated animals, mice treated with either moxifloxacin or imipenem showed significantly improved survival (P < 0.001). However, moxifloxacin treatment did not significantly alter the lethality induced by B. fragilis WAL R 13267 (ET(s)-moxifloxacin(s)-imipenem(s)) and E. coli (P = 0.078). Likewise, imipenem treatment showed no significant improvement of animals infected with B. fragilis RMA 5120 (ET(r)-moxifloxacin(s)-imipenem(r)) and E. coli (P = 0.074). This could possibly be explained by the fact that one mouse in the group treated with moxifloxacin died early after starting treatment and one mouse in the group treated with imipenem died during the injection with the antibiotic, probably accidentally. All other mice in the treatment groups who died after starting treatment apparently died because of the infection. There was no significant difference (P = 0.97) in the survival rate comparing the two treatment arms irrespective of infection with ET positive or ET negative strains. In addition, susceptibility or resistance of B. fragilis strains to moxifloxacin or imipenem had no impact on the survival rates.

In summary, moxifloxacin was as efficacious as imipenem in the treatment of severe systemic mixed aerobic/anaerobic infection in mice. With respect to the bacterial organ content, both therapeutic regimens could not always eliminate the inocula completely and there was a tendency that B. fragilis was more often recovered from the liver and kidneys of mice infected with ET positive B. fragilis strains (Table 2).

Discussion

In a previous similar experimental setting investigating the therapeutic efficacy of gentamicin, metronidazole and latamoxef, 65% of the infected mice receiving no treatment died up to day 9 post-infection whereas in our experiment, 44% of the infected mice without therapy died. This difference may be explained by the fact that in the earlier experiment, animal passaged B. fragilis was employed. Such a procedure now does not meet the approval of the regulatory agency. However, the mortality rate of 44% in our experiment is in accordance with the mortality rates reported by Onderdonk et al. (37%) and Brook & Gillmore (45%). The death of a few mice before the start of the antibiotic treatment in our experiment suggests that the infection was fully established before treatment with either moxifloxacin or imipenem was started.

Other studies with moxifloxacin were designed employing 50 and 100 mg/kg doses in mice that were estimated to be equivalent to 200 and 400 mg doses in human adults. Recent pharmacokinetic data indicated that in these experiments, moxifloxacin was underdosed.

Thus, our dosage regime was adjusted to 4 mg (~180 mg/kg) moxifloxacin daily and was administered in two separate doses. Also, our daily doses for imipenem are in accordance with recent published data using imipenem in the treatment of mixed-infection abscesses in mice. However, at the present time, unequivocal data are not available for moxifloxacin or imipenem concerning the daily dosage and frequency regimens in mice mimicking the therapeutic regimens in humans. Imipenem daily dosing regimens that took into account the short half-life of this antibiotic in mice varied from 384 to 1536 mg/kg per day given in 6–12 separate daily doses. With our mentioned dosage regimens, this study showed that moxifloxacin was as efficacious as imipenem in the treatment of severe systemic mixed aerobic/anaerobic infection in mice. However, the treatment courses with moxifloxacin or imipenem were not sufficient to sterilize the primary infection sites, namely the liver and kidneys in all instances.

This was also shown in a previous study comparing the efficacy of gentamicin, metronidazole and latamoxef for the treatment of mice infected with B. fragilis and E. coli. Imipenem is considered to be a standard therapeutic regimen in severe intra-abdominal infections and has served in this study as a reference. Our results with this treatment option are in accordance with previous investigations. Brook & Gillmore found a mortality rate of 45% in untreated mice in an intra-abdominal infection model caused by E. coli and B. fragilis. In the group treated with imipenem, none of the animals died. Nord & Lahnborg described an intra-abdominal infection model in rats. Eighty per cent of the
Mixed infection in mice treated with moxifloxacin

Table 1. Number of mice that died either before starting treatment (within day 1 post-infection) or after starting treatment (within day 2 to day 7 post-infection) and number of mice surviving until day 7 post-infection infected intravenously with different strains of *B. fragilis* and *E. coli* and treated with moxifloxacin or imipenem/cilastatin

<table>
<thead>
<tr>
<th>Infection with</th>
<th><em>B. fragilis</em> (ET(-)-MXF(s)-IMP(r)) + E. coli</th>
<th><em>B. fragilis</em> (ET(-)-MXF(s)-IMP(s)) + E. coli</th>
<th><em>B. fragilis</em> (ET(+)-MXF(s)-IMP(s)) + E. coli</th>
<th><em>B. fragilis</em> (ET(+)-MXF(r)-IMP(s)) + E. coli</th>
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<td>untreated  MXF  IMP</td>
<td>untreated  MXF  IMP</td>
<td>untreated  MXF  IMP</td>
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<td>No. of animals infected</td>
<td>20  20  20</td>
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<td>No. of animals dead before starting treatment</td>
<td>1  1  2</td>
<td>1  1  1</td>
<td>1  1  1</td>
<td>1  1  1</td>
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<td>No. of animals dead after starting treatment</td>
<td>7  1  0</td>
<td>8  0  3</td>
<td>9  2  1</td>
<td>6  2  1</td>
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<td>Total of animals surviving (day 7 post-infection)</td>
<td>12  18  18</td>
<td>11  19  16</td>
<td>10  17  18</td>
<td>12  18  18</td>
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MXF, moxifloxacin; IMP, imipenem/cilastatin.
Breakpoints: moxifloxacin ≥ 4 mg/L; imipenem ≥ 8 mg/L.
*B. fragilis* (ET(-)-MXF(s)-IMP(r)): *B. fragilis* RMA 0309 (enterotoxin negative, moxifloxacin susceptible, imipenem resistant).
*B. fragilis* (ET(-)-MXF(s)-IMP(s)): *B. fragilis* RMA 5120 (enterotoxin negative, moxifloxacin and imipenem susceptible).
*B. fragilis* (ET(+)-MXF(s)-IMP(s)): *B. fragilis* WAL R 13267 (enterotoxin positive, moxifloxacin resistant, imipenem susceptible).
*B. fragilis* (ET(+)-MXF(r)-IMP(s)): *B. fragilis* RMA 6791 (enterotoxin positive, moxifloxacin and imipenem susceptible).
*E. coli* strain susceptible to both moxifloxacin and imipenem.
Table 2. Bacterial organ contents of surviving mice killed at day 7 post-infection infected intravenously with different strains of *B. fragilis* and *E. coli* and treated with moxifloxacin or imipenem/cilastatin

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<tr>
<th>Infection with</th>
<th>B. fragilis (ET((+-))-MXF(\geq)-IMP(\geq)) + E. coli</th>
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<th>B. fragilis (ET((+-))-MXF(\geq)-IMP(\geq)) + E. coli</th>
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<td>No. of animals with <em>E. coli</em> organ content (cfu) of</td>
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<td>No. of animals with <em>B. fragilis</em> organ content (cfu) of</td>
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MXF, moxifloxacin; IMP, imipenem/cilastatin.
Breakpoints: moxifloxacin ≥ 4 mg/L; imipenem ≥ 8 mg/L.
*B. fragilis* (ET\((+-)\)-MXF\(\geq\)-IMP\(\geq\)): *B. fragilis* RMA 539 (enterotoxin negative, moxifloxacin susceptible, imipenem resistant).
*B. fragilis* (ET\((+-)\)-MXF\(\geq\)-IMP\(\geq\)): *B. fragilis* RMA 5120 (enterotoxin negative, moxifloxacin and imipenem susceptible).
*B. fragilis* (ET\((+-)\)-MXF\(\geq\)-IMP\(\geq\)): *B. fragilis* RMA 6791 (enterotoxin positive, moxifloxacin and imipenem susceptible).
*B. fragilis* (ET\((+-)\)-MXF\(\geq\)-IMP\(\geq\)): *B. fragilis* WAL R 13267 (enterotoxin positive, moxifloxacin resistant, imipenem susceptible).
*E. coli* strain susceptible to both moxifloxacin and imipenem.
untreated animals died, whereas all the animals treated with imipenem survived. In an additional study, Nord & Lahmborg compared the efficacy of biapenem and imipenem. Again, in this study 80% of the untreated rats died, but only two out of 40 animals in the two treatment arms.

Moxifloxacin is a new quinolone that, like trovafloxacin or clinafloxacin, belongs to the fluoroquinolone group IV as defined by Naber & Adam. As a result of the in vitro anaerobe activity of group IV quinolones, it was of interest to evaluate whether moxifloxacin with its documented in vitro activity against aerobic and anaerobic bacteria could also show efficacy in an animal model of mixed aerobic/anaerobic infection.

To study the therapeutic efficacy of moxifloxacin, we used B. fragilis strains either susceptible or resistant to moxifloxacin, and ET positive or negative. ET of B. fragilis might be a virulence factor even in diseases other than diarrhoea since ET positive B. fragilis strains are also regularly found among blood culture isolates. In our experiments there was no significant difference in the outcome of the mice infected with ET positive or ET negative B. fragilis strains irrespective of the treatment regimen. This underscores earlier observations by Onderdonk et al. that E. coli alone is primarily responsible for the lethal effects in the animals and B. fragilis antigens increase the susceptibility of the animals to E. coli lipopolysaccharide. There was a tendency that B. fragilis was more often recovered from the liver and kidneys of mice infected with ET positive B. fragilis strains. This supports the hypothesis that ET of B. fragilis might be a virulence factor in diseases other than diarrhoea.

Moxifloxacin and imipenem were equally efficacious in reducing infectious lethality in animals. Resistance of the B. fragilis strains to a particular treatment regimen did not impact on treatment results. Although in vitro kill kinetics suggest that imipenem can decimate a B. fragilis inoculum more rapidly than moxifloxacin, no significant differences in eradication rates were observed in the surviving animals. The results indicate that the course of therapy selected here was too short, since not all surviving animals were sterilized from the infectious agents. However, technical reasons (thrombosis) often prohibit longer intravenous treatments. In a recently published abstract, we showed in an in vitro pharmacokinetic/pharmacodynamic (PK/PD) model in single and mixed culture, that moxifloxacin was only bacteriostatic but not bactericidal against the B. fragilis strains investigated in this study. These findings might explain the abscess formation of B. fragilis after treatment and the survival of mice irrespective of the susceptibility to moxifloxacin or imipenem. In the in vitro PK/ PD model, moxifloxacin was fully bactericidal against E. coli. This might explain the significantly improved survival rate after moxifloxacin treatment in the infected mice.

References


