Tissue penetration of moxifloxacin into human gallbladder wall in patients with biliary tract infections

Michael C. Ober1, Torsten Hoppe-Tichy1, Jörg Königer2, Oliver Schunter3, Hans-Günther Sonntag4, Markus A. Weigand5, Jens Encke6, Carsten Gutt2 and Stefanie Swoboda1*

1Pharmacy Department, University Hospital of Heidelberg, Im Neuenheimer Feld 670, D-69120 Heidelberg, Germany; 2Department of Surgery, University Hospital of Heidelberg, Im Neuenheimer Feld 110, D-69120 Heidelberg, Germany; 3Hospital Bietigheim-Bissingen, Riedstr. 12, D-74321 Bietigheim-Bissingen, Germany; 4Department of Medical Microbiology and Hygiene, Institute of Hygiene, University of Heidelberg, Im Neuenheimer Feld 324, D-69120 Heidelberg, Germany; 5Department of Anaesthesiology, University Hospital of Heidelberg, Im Neuenheimer Feld 110, D-69120 Heidelberg, Germany; 6Department of Gastroenterology and Hepatology, University Hospital of Heidelberg, Im Neuenheimer Feld 410, D-69129 Heidelberg, Germany

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Objectives: Moxifloxacin, the newest fourth-generation fluoroquinolone, has a broad spectrum of antibacterial activity covering both Gram-positive and Gram-negative aerobic and anaerobic bacteria and is therefore very well suited for the treatment of biliary tract infections. The present study aimed to determine the penetration of moxifloxacin into gallbladder tissue to evaluate its antibiotic potential in this indication.

Patients and methods: Hospitalized patients with acute cholecystitis received a single, 1 h infusion of 400 mg of moxifloxacin before cholecystectomy. Serum and gallbladder wall tissue samples were collected during surgery, and the moxifloxacin concentrations were measured by HPLC.

Results: Sixteen patients (eight men and eight women) were included between January 2007 and April 2008. The time between start of infusion and gallbladder removal ranged from 50 min to 21 h 10 min. The serum concentration at the time of cholecystectomy was between 0.39 and 4.37 mg/L, and the tissue concentration between 1.73 and 17.08 mg/kg. The tissue-to-serum concentration ratio ranged from 1.72 to 6.33.

Conclusions: The results show that moxifloxacin penetrates well into gallbladder tissue and is therefore a therapeutic option for biliary tract infection. The highest concentrations in serum and gallbladder tissue were measured shortly after the end of a 1 h infusion. As perioperative prophylaxis, moxifloxacin should therefore be administered 30–60 min before the first surgical incision.

Keywords: tissue concentration, perioperative prophylaxis, surgery

Introduction

One of the most common acute diseases in the Western world is acute cholecystitis, which may range from a mild, painful disorder to a life-threatening illness due to complications. It is usually caused by infection in an obstructed bile system, associated with gallbladder stones in >90% of cases.1,2 Gram-negative bacteria, mainly Enterococcus coli and Klebsiella spp. (together >50%), are the most frequent causative pathogens. Gram-positive bacteria, especially enterococci, and anaerobic bacteria (mainly Bacteroides spp. and Clostridium spp.) are also isolated; 30%–80% of cases are caused by more than one pathogen. The pathogenicity of enterococci in this condition is still unclear, as they are almost always detected in mixed infections.1

Antibiotic therapy and laparoscopic cholecystectomy are the most common approach to the treatment of cholecystitis. The choice of antimicrobial therapy is based on various criteria, including identification of the most common pathogens, the spectrum of the antimicrobial agent, and its pharmacokinetic...
Table 1. MIC$_{90}$s of moxifloxacin for the most common pathogens in biliary tract infection

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>MIC$_{90}$ (mg/L)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-negative bacteria</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>0.06</td>
</tr>
<tr>
<td><em>Klebsiella</em> spp.</td>
<td>1.0</td>
</tr>
<tr>
<td><em>Enterobacter</em> spp.</td>
<td>0.5</td>
</tr>
<tr>
<td>Gram-positive bacteria</td>
<td></td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>1.0</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>8.0</td>
</tr>
<tr>
<td>Anaerobes</td>
<td></td>
</tr>
<tr>
<td><em>Bacteroides fragilis</em></td>
<td>1.0</td>
</tr>
<tr>
<td><em>Clostridium</em> spp.</td>
<td>1.0</td>
</tr>
</tbody>
</table>

$^a$Reported by Edmiston et al.$^4$

and pharmacodynamic properties. The drug chosen must attain concentrations clearly above the MIC$_{90}$s for the pathogens at the site of infection. Tissue penetration is therefore an important selection criterion. Table 1 shows the usual MIC$_{90}$s of moxifloxacin for common pathogens in biliary tract infections. *Enterococcus faecium* is less susceptible than *Enterococcus faecalis* with an MIC$_{90}$ of up to 8 mg/L, and it therefore shows intermediate resistance. Most anaerobic bacteria have an MIC$_{90}$ between 1 and 4 mg/L.$^{3,4}$

Drugs chosen to treat cholecystitis must have adequate bactericidal activity against all common pathogens and also penetrate sufficiently into gallbladder tissue. Since the bactericidal effect of moxifloxacin is concentration dependent, the maximum concentration ($C_{\text{max}}$) should be significantly higher than the MIC$_{90}$ to achieve appropriate activity.$^5$

Moxifloxacin, the newest fourth-generation fluoroquinolone (Avalox$^8$; Bayer Vital GmbH, Leverkusen, Germany), meets all these requirements. It has excellent activity against the common pathogens found in gallbladder infection (except *E. faecium*) and shows good penetration into various tissues.$^{4,6,7}$ It also reaches high concentrations in bile, which are, however, significantly reduced in patients with biliary obstruction.$^8$

The aim of the present study was to determine serum and tissue concentrations of moxifloxacin in patients undergoing laparoscopic cholecystectomy after perioperative intravenous (iv) antimicrobial prophylaxis with a single infusion of 400 mg, and to establish whether it achieves concentrations in gallbladder tissue clearly above the MIC$_{90}$s for the most common pathogens in biliary tract infections.

Materials and methods

Study design

Three hospitals participated in the present study focusing on hospitalized patients with acute cholecystitis. Before laparoscopic cholecystectomy, they received a single infusion of 400 mg of moxifloxacin as perioperative prophylaxis.

The study protocol was approved by the independent ethics committee of the University Hospital of Heidelberg in 2006, and the ethics committees of the participating hospitals and the German health authority (BfArM). It was conducted in accordance with Good Clinical Practice, the Declaration of Helsinki and German federal guidelines. Written informed consent was obtained from all patients enrolled. Serum and tissue samples were collected from patients enrolled between January 2007 and April 2008 to determine moxifloxacin concentrations.

Before surgery, patients were to receive a single, 250 mL infusion of 400 mg of moxifloxacin over 60 min.

Serum and tissue samples

At the time the gallbladder was removed, blood samples were collected in serum tubes, centrifuged to obtain serum and stored at $-70^\circ$C until analysis. Tissue samples were collected after cholecystectomy and also stored at $-70^\circ$C until analysis. The time between start of infusion and gallbladder removal and serum collection ranged from 50 min to 21 h 10 min with a mean of 6 h 8 min and a median of 4 h 17 min.

Analytical method

Moxifloxacin was determined by HPLC with fluorimetric detection according to the method of Stass and Dalhoff$^9$ The analysis was performed in the laboratory of the Pharmacy Department of the University Hospital of Heidelberg.$^7$

Calibration standards for tissues were prepared as described previously.$^{10}$

The analytical method was validated according to the Food and Drug Administration (FDA) Guidance for Industry—Bioanalytical Method Validation and provided good validation data for accuracy and precision (quality control samples). The assay was linear for serum over the concentration range 0.05–8.0 mg/L and for tissue samples for 0.5–20.0 mg/kg. The accuracy of the serum standard curve was 96.6–103.7%, and for the tissue standard curve 93.9%–105.9%. The inter-day coefficient of variation ranged from 1.53% to 6.23% for low, middle and high concentrations (0.1, 1.0 and 4.0 mg/L). The limit of quantification was 0.05 mg/L for serum samples and 0.5 mg/kg for tissue samples. No interfering peaks were observed in the assay. Cross-validation against external controls (Bayer AG, Leverkusen, Germany) was carried out successfully.

Statistics

Serum and tissue concentrations were plotted against the time between start of infusion and gallbladder removal and serum and tissue sample collection using Origin Pro 8 SRO (OriginLab Corporation, Northampton, MA, USA). Mann–Whitney $U$-tests were used to detect significant correlations between serum and tissue concentrations and patient characteristics. Covariance analysis was performed using SAS (SAS Institute Inc., Cary, NC, USA). The significance level was set at $P<0.05$.

Results

Patients

Sixteen patients (eight male and eight female) were enrolled. The age ranged from 24 to 76 years with a mean of 58.5 years (SD $\pm$ 15.8). The body mass index (BMI) ranged from 22.6 to 36.0 kg/m$^2$ with a mean of 28.8 kg/m$^2$ (SD $\pm$ 3.44).

All 16 patients received 400 mg of moxifloxacin before surgery as a 1 h infusion. It was continued after surgery in eight
patients for 1 day. No further antibiotics were given. None of the 16 patients developed post-operative infections.

Adverse events were observed in four patients. One developed haematuria 2 days after surgery, and a wound healing disorder was diagnosed in a further patient 1 week after surgery, thus primary healing was delayed by 7 days. The third patient complained of upper abdominal pain 17 days after the end of moxifloxacin treatment. The fourth developed an allergy with pharyngeal swelling 1 day after surgery and overflow incontinence due to adenoma of the prostate 2 days after surgery. The wound healing disorder and the allergy were considered by the investigator to be probably related to moxifloxacin.

Moxifloxacin concentrations

Moxifloxacin was administered to the 16 patients between 50 min and 21 h 10 min before gallbladder removal (median 4 h 17 min). Serum concentrations of moxifloxacin of between 0.39 and 4.37 mg/L were measured, reflecting the time lag between start of infusion and surgery. Gallbladder tissue showed moxifloxacin concentrations of between 1.73 and 17.08 mg/kg, also reflecting the time lag. The tissue-to-serum ratio ranged from 1.72 to 6.33 (mean $3.00 \pm 1.39$, median 2.39).

Figures 1 and 2 show the serum and tissue concentrations plotted against the time lag between start of infusion and sampling. Figure 3 shows the tissue-to-serum ratio plotted against the time lag.

The most important influence on the concentrations of moxifloxacin in serum and tissue samples was the time lag between the start of infusion and gallbladder removal: the correlation between serum ($P<0.0001$) and tissue concentrations ($P=0.0003$) and the time lag was statistically significant. When the time lag was included, covariance analysis did not show any statistically significant correlations between gender and moxifloxacin concentrations.

The correlations between BMI and serum and tissue concentrations were analysed with a covariance test including different time lags between start of infusion and removal of gallbladder.

There was no statistically significant correlation between tissue ($P=0.1818$) or serum concentration ($P=0.7780$) and BMI. However, the tissue-to-serum ratio correlated significantly with BMI ($P=0.0471$) showing that a greater BMI was associated with a higher tissue-to-serum ratio (Figure 4).

Moxifloxacin concentration with regard to MIC$_{90}$

The most frequent pathogens in gallbladder infections ($E. coli$ and $Klebsiella$ spp., together responsible for >50% of biliary tract infections) have an MIC$_{90}$ < 1.0 mg/L. To achieve activity against anaerobes, the antimicrobial concentration should exceed 2 mg/L as moxifloxacin kills most anaerobic strains at $\leq$ 2 mg/L.$^{11}$

Fourteen of the 16 patients had tissue concentrations exceeding 2 mg/kg at the time of surgery. Only two patients with very long time lags between start of infusion and surgery (16.08 and
Discussion

To establish whether an antibacterial agent is appropriate for use in a specific type of infection, the concentration of the drug at the site of infection must be determined because the blood concentration may not give reliable information on clinical efficacy. The main objective of the present study was to determine tissue concentrations of moxifloxacin in the gallbladder wall to establish whether adequate tissue concentrations for the treatment of biliary tract infections are reached. To be suitable for treatment, the moxifloxacin tissue concentrations would have to exceed the MIC90s for the most common causative pathogens. To our knowledge, this is the first study describing moxifloxacin concentrations in gallbladder tissue in human patients.

The serum concentrations of moxifloxacin we found were similar to those recently reported.12 Longer time lags between the start of infusion and surgery were associated with significantly lower serum and tissue concentrations of moxifloxacin. Although this was expected, our findings confirm standard guidelines for perioperative prophylaxis in surgery, indicating that the best time to start antibiotic prophylaxis is between 30 and 60 min before the first surgical incision.13,14

Moxifloxacin had a half-life of ~7 h in the present study. Previous studies have, however, shown a longer half-life of 8.2–15.4 h after iv administration.15,16 There are several explanations for the shorter half-life we observed. This has been observed in other trials, and corresponds to the dominant half-life within 12–24 h after the first dose, which is shorter than the half-life in the steady state.17–19 Our study contained bias in that we generated more data in the first 12 h from the start of infusion to sampling because medical and ethical reasons prevented us from arbitrarily delaying surgery. A further factor is that we had only one sampling point per patient, and were therefore only able to calculate the half-life for the population and not for each individual patient, as in a classic Phase I study. The inter-individual pharmacokinetic variability therefore had a considerable influence in obtaining the half-life of 7 h.

As expected, the serum and tissue concentrations were not gender dependent. Our findings suggest that a greater BMI is associated with a higher tissue-to-serum concentration ratio. In a previous study, we made a similar observation with levofloxacin in cholecystectomy patients with high BMIs. A possible explanation could be the high partition coefficient of moxifloxacin with its tendency to distribute into adipose tissue. Its clinical relevance is questionable.

A possible limitation of this study is that total, and not unbound, moxifloxacin was assessed in serum and tissue samples. Moxifloxacin protein binding values of 42% + 8% have been reported.16 Thus, even if half of the total moxifloxacin in gallbladder tissue is unbound, the concentrations in tissue attained in this study (except for cases with very long time lags between administration and surgical intervention) were still higher than the MIC90s reported for pathogens commonly isolated in patients with biliary tract infection, including anaerobes.20

It is noteworthy that none of the patients in our small sample developed post-operative infections. Whether such an excellent result could be maintained in a larger number of patients in the everyday clinical setting is questionable.

Concluding from the described results the question can be raised of whether moxifloxacin could be an alternative to other advised antibiotic agents in biliary tract infections. The major argument for the use of moxifloxacin in biliary tract infections is its broad spectrum of activity. None of the other antibiotics recommended has such a broad spectrum, especially with regard to anaerobic bacteria. The cephalosporins, for example, are not active against Bacteroides spp., Clostridium spp. or enterococi. Anaerobic activity is a prerequisite for the treatment of patients with an expected mixed infection, such as elderly patients who have already undergone biliary tract surgery or critically ill patients. Although ciprofloxacin and levofloxacin also achieve good tissue concentrations, they are also not active against anaerobes.5,21 Moxifloxacin therefore has a broader spectrum of activity than other antibiotics commonly used to treat biliary tract infections and shows excellent penetration into the gallbladder wall.

Many antibiotics, also fluoroquinolones, can cause side effects affecting the liver. The possible hepatotoxicity is also reflected in the latest European Medicines Agency (EMEA) recommendation of July 2008 for the use of oral moxifloxacin. Considering that patients with cholecystitis may have a degree of biliary stasis, it is essential to check all hepatic laboratory values before and during moxifloxacin therapy and to discontinue treatment if signs and symptoms of fulminant hepatic disease develop such as rapidly developing asthenia associated with jaundice.

Conclusions

After single iv dosing in cholecystectomy patients, moxifloxacin achieves concentrations in serum and the gallbladder wall adequate to kill the most common pathogens in cholecystitis. It should be administered 0.5–1 h before the first surgical incision because peak serum and tissue concentrations higher than the

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Figure 4. Correlation between tissue-to-serum ratio of moxifloxacin and BMI in 16 patients with biliary tract infections after a single iv dose of 400 mg.

21.17 h) did not achieve such concentrations. Eleven of the 16 patients had serum concentrations exceeding 2 mg/L at the time of gallbladder removal.
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relevant MIC90s occur shortly after the end of a 1 h infusion. Moxifloxacin therefore fulfills the pharmacokinetic and pharmacodynamic requirements for the treatment of biliary tract infections. The clinical outcome in this indication should be evaluated in randomized clinical trials.

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Transparency declarations

None to declare.

References