Experimental pneumococcal pleural empyema model: the effect of moxifloxacin

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Objectives: Pleural empyema is a serious complication of pneumonia, the optimal therapy of which is still unknown. The objective of this study was to evaluate the use of moxifloxacin in this condition.

Methods: Pleural empyema was induced in rabbits by intrapleural administration of Pasteurella multocida (10^5–6 cfu) or turpentine (0.3 mL) followed 3 h later by instillation of Streptococcus pneumoniae (ATCC 49619) (10^6 cfu) into the pleural cavity. The MICs of moxifloxacin for S. pneumoniae and P. multocida were 0.4 and 0.05 mg/L, respectively. Starting 30 h following S. pneumoniae challenge intramuscular moxifloxacin 12.5 and 25 mg/kg was administered × 4 (every 12 h). Pleural empyema fluid samples were obtained for bacterial count at 12 h intervals following the first three moxifloxacin administrations. Moxifloxacin levels in pleural empyema and serum samples were obtained at 0, 30, 60, 120, 240, 360 and 480 min and 12 h after the 4th dose and determined by bioassay.

Results: In control animals, S. pneumoniae (and P. multocida) persisted in the pleural empyema. S. pneumoniae also persisted in the pleural empyema fluid when moxifloxacin was administered at 12.5 mg/kg (×4 administrations). Mean serum and pleural empyema peak moxifloxacin levels (following the 25 mg/kg dose) were 7.6 (±3.2) and 4.8 (±2.5) mg/L, respectively. Pleural empyema peak moxifloxacin concentration lagged 1 h after serum moxifloxacin. Serum and pleural empyema half-lives were ∼1.5 and ∼6 h, respectively. Serum AUC1–12 was 29.4 (±6.8) mg·h/L and serum area under the inhibitory concentration curve (AUIC) was 73.5 mg·h/L. Pleural empyema AUC1–12 was 34.3 (±11.7) mg/L and pleural empyema AUIC was 85.8 mg·h/L. S. pneumoniae was eradicated from pleural empyema following a single dose of moxifloxacin 25 mg/kg in 52% of the animals and in 96% following four doses. Moxifloxacin was also effective in eradication of P. multocida. The rate of pleural empyema sterilization was related to moxifloxacin serum AUIC (r = 0.82) as well as serum peak moxifloxacin level (r = 0.84), but not to pleural empyema AUIC (r = 0.19) or pleural empyema peak levels. The results were similar for both methods of induction of pleural empyema.

Conclusions: Moxifloxacin appears to penetrate well into experimental pleural empyema and effectively sterilize it from S. pneumoniae. Sterilization of S. pneumoniae is related to serum AUIC rather than to moxifloxacin pharmacokinetics in pleural empyema.

Keywords: moxifloxacin, Pasteurella multocida, pleural empyema, Streptococcus pneumoniae, turpentine

Introduction

Pleural empyema is a serious complication of pneumonia, affecting ∼1–3% of patients with pneumococcal pneumonia. Some believe that adequate levels of antibiotics are achieved in empyema fluid only in the early exudative phase, when the visceral and parietal pleura are freely movable, and there are not yet any pleural adhesions forming pockets of and loculations of pus, and when the fluid has a low viscosity with a low number of white blood cells (WBCs). However, in a later
stage, called the fibropurulent or transitional phase, because of the thickness of the pleura and the unique physicochemical characteristics of the empyema fluid, consisting of high viscosity, high WBC count, pH −7.0 to −7.29, lactate dehydrogenase 500–1000 IU and glucose content 40–60 mg/dL, the penetration of antibiotics into pleural empyema and their effectiveness in these circumstances may vary between antibiotics.3 In the organizing or chronic phase when the pleura is converted into a fibrous tissue and the remaining fluid is purulent, highly viscous with pH < 7.0, the penetration and efficacy of most antibiotics is probably non-existent.3,4

Moxifloxacin is a fluoroquinolone that is efficacious against Streptococcus pneumoniae and other microorganisms causing pleural empyema, is widely distributed in tissues5 and could have an important role in the treatment of pleural empyema caused by S. pneumoniae.

The aim of this study was therefore to evaluate moxifloxacin in a rabbit model of pneumococcal pleural empyema.

Materials and methods

Animals

New Zealand white male rabbits weighing 2–3 kg were obtained from Lowenstein Farms, Yokneam, Israel. Animals were housed in individual cages and allowed food and water ad libitum.

This research adhered to the ‘Principles of Laboratory Animal Care’ of the Tel Aviv University, Faculty of Medicine and was supervised by the hospital’s veterinarian. The study protocol was approved by the Sheba Medical Center Animal Care Committee (Animal Helsinki Committee).

Bacteria

Pasteurella multocida isolated from a human bite wound (by a dog) was used for priming. S. pneumoniae used was ATCC 49619. MICs for S. pneumoniae and P. multocida were determined by the broth microdilution method, with Mueller–Hinton broth supplemented with divalent cations and 5% lysed horse blood. The MICs of moxifloxacin for S. pneumoniae and P. multocida were 0.4 and 0.05 mg/L, respectively.

Pleural empyema induction

Two different models of pleural empyema induction were used, as described previously.6–8 In the first induction method, P. multocida (105–6 cfu) in a final volume of 0.3 mL was injected intrapleurally through the cannula (vide infra) as a priming step, into a group of 26 animals. In the second mode of induction of pleural empyema, turpentine (0.3 mL) was administered into the pleural source of a group of 26 animals via the intrapleural catheter. Three hours later washed S. pneumoniae (109 cfu) (in a final volume of 0.2 mL saline) were instilled through the cannula into the pleural cavity of animals who underwent induction in either method.

A rubber stopper-capped 16 gauge cannula was inserted into the pleural cavity of the rabbits under ketamine (Ketalar, Parke, Davis & Co., Israel) and xylazine (Rompun; Bayer AG, Leverkusen, Germany) anaesthesia, as described previously.6,7 The rubber cap was secured subcutaneously, facilitating injection of both bacterial strains and an irritant into the pleural space as well as aspiration of empyema fluid samples for analysis.

Antibiotic administration

Starting 30 h following S. pneumoniae intrapleural instillation, moxifloxacin 12.5 and 25 mg/kg were administered × 4 (every 12 h) intramuscularly to imitate human pharmacokinetics of moxifloxacin.

The following groups of animals were used. For moxifloxacin 12.5 mg/kg: 15 animals were used, in seven empyema was induced by turpentine and in eight rabbits by P. multocida injection. In the moxifloxacin 25 mg/kg group: in 13 animals pleural empyema was induced by turpentine and in 12 by P. multocida. Six animals in each priming group served as controls and were not treated.

Pleural fluid and serum specimens

Pleural empyema fluid samples (0.25 mL of each sample) were obtained for bacterial count at 12 h intervals following the first three moxifloxacin administrations. Moxifloxacin levels in pleural empyema and serum samples (0.1 mL each) were obtained at 0, 30, 60, 120, 240, 360 and 480 min and 12 h after the 4th dose. In control animals, pleural empyema was induced but not treated.

Analyses

Pleural empyema pH, glucose and protein were measured with urine dipsticks (Medi-Test Combi 9, Macherey-Nagel, Duren, Germany).

Moxifloxacin (Bayer-AG) concentrations in serum and fluid were measured microbiologically using Bacillus subtilis (ATCC 6633) on Trypticase soy agar pH 9.0.

For serum moxifloxacin determinations, moxifloxacin was diluted in pooled rabbit serum containing no antibiotics, and for pleural empyema determinations in normal saline pH 7.4 at a concentration of 0.15–20.0 mg/L. The sensitivity of the method was 0.08 mg/L, the detection limit 0.15 mg/L and day-to-day variation 12%.

All measurements were carried out in duplicate (when the difference between the results obtained exceeded 50%, another set of measurements was carried out and the results averaged).

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Areas under the time–concentration curves (AUCs) over 12 h (AUC$_{0-12}$) for moxifloxacin were calculated with the trapezoidal rule. AUC/MIC ratios, defined as the area under the inhibitory concentration curve (AUIC) were calculated by dividing the AUC$_{0-12}$ by the MIC for the specific strain. AUC$_{0-24}$ was defined as twice the AUC$_{0-12}$.

Pathological studies

From each experimental group, pleural tissues from four animals were subjected to autopsy and histological studies. Pathology revealed thickened visceral and parietal pleural surfaces with thick purulent exudate and multiple adhesions forming pus pockets. Histological sections stained with haematoxylin–eosin (magnification ×100 and ×400) showed prominent acute inflammatory cell infiltration (Figure 1).

Statistical analysis

Results are expressed as average ± S.D. The strength of association between pharmacodynamic parameters and eradication was evaluated with Pearson’s exact test.

Table 1. Pharmacokinetic parameters of moxifloxacin (MXF) in serum and pleural empyema in the rabbit

<table>
<thead>
<tr>
<th>MXF (mg/kg)</th>
<th>Peak (mean; mg/L)</th>
<th>AUC$_{0-24}$ (mg·h/L)</th>
<th>AUIC$_{0-24}$ (mg·h/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>serum pleural empyema</td>
<td>serum pleural empyema</td>
<td>serum pleural empyema</td>
</tr>
<tr>
<td>12.5</td>
<td>3.4 2.4</td>
<td>19.2 38.6</td>
<td>48.0 96.5</td>
</tr>
<tr>
<td>25.0</td>
<td>7.6 4.8</td>
<td>58.8 68.6</td>
<td>147.0 171.5</td>
</tr>
</tbody>
</table>

Figure 1. Histological section of pleura after turpentine induction showing prominent acute inflammatory infiltration (haematoxylin–eosin; magnification ×100 and ×400).

Results

In untreated controls, 3/12 animals survived *S. pneumoniae* challenge. In these animals *S. pneumoniae* (and *P. multocida*) persisted in the pleural empyema for >7 days.

Pleural empyema before moxifloxacin treatment was characterized by being purulent containing: protein > 500 mg/mL; glucose (mean) 88 mg/dL (range 50–150 mg/dL); mean pH 7.69; pH 8.13 in turpentine-treated animals and 7.3 in the *P. multocida* model. These parameters did not change significantly during moxifloxacin therapy.

In 15 animals treated with moxifloxacin 12.5 mg/kg, mean serum and pleural empyema peak moxifloxacin levels were 3.4 (±1.2) and 2.4 (±1.6) mg/L, respectively (Table 1). Serum AUC$_{0-12}$ was 9.6 (±5.3) mg·h/L and serum AUIC was 24.0 mg·h/L. Pleural empyema AUC$_{0-12}$ was 19.3 (±9.9) mg·h/L and pleural empyema AUIC was 48.3 mg·h/L (Table 1, Figure 2). Mean *S. pneumoniae* count in the pleural empyema did not change significantly during moxifloxacin administration, ranging from $6.5 \times 10^6$ (±$8 \times 10^6$) cfu/mL before moxifloxacin administration to $4.3 \times 10^6$ (±$9.8 \times 10^6$) cfu/mL after the 4th dose.

From three animals, no drug level measurements were available. In 22 animals treated with moxifloxacin 25 mg/kg, mean serum and pleural empyema peak moxifloxacin concentrations were 7.6 (±3.2) and 4.8 (±2.5) mg/L, respectively (Table 1). Pleural empyema peak moxifloxacin concentration lagged 60–90 min after serum peak concentration, which occurred at 30–60 min (Figure 3). Moxifloxacin serum and pleural empyema $t_{1/2}$ values were ∼1.5 and ∼6 h, respectively.

Serum AUC$_{0-12}$ was 29.4 (±6.8) mg·h/L and serum AUIC was 73.5 mg·h/L. Pleural empyema AUC$_{0-12}$ was 34.3 (±11.7) mg·h/L and pleural empyema AUIC was 85.8 mg·h/L (Table 1).

*S. pneumoniae* was eradicated from pleural empyema following the first 25 mg/kg moxifloxacin dose in 52% of the animals and following four moxifloxacin doses in 96% (Table 2). Moxifloxacin was also efficacious in *P. multocida* eradication (Table 2).

The rate of pleural empyema sterilization was significantly related to moxifloxacin serum AUC ($r = 0.82$) as well as to serum peak moxifloxacin level ($r = 0.84$), but not to pleural...
empyema AUC \( (r = 0.19) \) or pleural empyema peak moxifloxacin levels \( (r = 0.26) \). There were no significant differences in pharmacokinetic and pharmacodynamic parameters between the two models of pleural empyema induction (data not shown).

Discussion

Our two experimental models of acute pleural empyema in the rabbit suggest that pleural empyema caused by \( S. \) pneumoniae can be induced in the rabbit by prior instillation of turpentine as well as by priming both the visceral and parietal pleurae with intrapleural \( P. \) multocida. In both methods a pleural inflammatory reaction that facilitated \( S. \) pneumoniae infection was induced. Pleural empyema could not be induced in the rabbit by direct intrapleural instillation of several serotypes of \( S. \) pneumoniae alone. In smaller animals (mice and rats), the two pleural cavities are not separated and thus induction of empyema that necessitates the creation of prior pneumothorax does not allow the animals to survive long enough for pleural empyema to develop. A model has been developed in the rabbit showing that the pleural cavity can be primed with \( P. \) multocida, which is a natural pathogen for the rabbit, and a subsequent instillation of \( S. \) pneumoniae will induce a pleural empyema from which both strains can be consistently isolated.\(^7\)

Following \( S. \) pneumoniae instillation into the primed pleural space, the fluid secreted in both models was purulent and the pleural surfaces in the experimental animals were highly inflamed, similar to the situation in human pleural empyema.

Moxifloxacin at 25 mg/kg produced pleural empyema concentrations in all animals that were adequate for a rapid sterilization (after the 1st dose in 52%, after the 2nd dose in 84% and after the 4th dose in 96% of the animals) of the pleural empyema from \( S. \) pneumoniae (and from \( P. \) multocida).\(^7\)

AUIC is an important pharmacodynamic parameter that predicts fluoroquinolone therapeutic success.\(^9\) We found that the rate of sterilization of the pleural empyema was significantly related to moxifloxacin serum AUC. The AUIC obtained (based on AUC\(_{0-12}\) measured in our experiments) was \( \sim 145 \) mg·h/L, far above the accepted cut-off of 30 mg·h/L necessary for clinical and microbiological success for eradication of uncomplicated \( S. \) pneumoniae infections.\(^9\) Moreover, in animals that received moxifloxacin 12.5 mg/kg, serum AUIC was 48 mg·h/L. \( S. \) pneumoniae persisted in the pleural empyema throughout the experiment, suggesting that a cut-off serum AUIC of 30 mg·h/L is insufficient for the treatment of pleural empyema caused by \( S. \) pneumoniae at least in this model. The reasons for this discrepancy may be due to the physico-chemical characteristics of the empyema fluid, which decrease the bacterial killing effect, and to the adhesions in the pleural cavity, which caused pockets of pus that might have prevented adequate penetration of moxifloxacin to the foci of infection.

In the present study, antibiotic treatment was initiated relatively early in the course of empyema, 30 h after bacterial challenge. However, even at this early period, we observed thick pleura, pockets of pus and purulent pleural empyema.

<table>
<thead>
<tr>
<th>No. of doses of MXF(^a)</th>
<th>( S. ) pneumoniae</th>
<th>( P. ) multocida</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>52 (13/25)</td>
<td>29 (2/7)</td>
</tr>
<tr>
<td>2</td>
<td>84 (21/25)</td>
<td>71 (5/7)</td>
</tr>
<tr>
<td>3</td>
<td>92 (23/25)</td>
<td>100 (7/7)</td>
</tr>
<tr>
<td>4</td>
<td>96 (24/25)</td>
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</table>

\(^a\)Dosage MXF, 25 mg/kg.
fluid, similar to the findings in the fibropurulent and the beginning of the organizing-chronic stage of human pleural empyema.4,10

The repeated pleural fluid aspirations carried out in the present investigation could possibly be regarded as a partial pleural empyema drainage facilitating recovery. Therefore we cannot conclude that cure was achieved by moxifloxacin alone. However, it resembles more closely the current clinical approach to pleural empyema.

The pH values measured in the present study were higher than in previously described similar experiments where the pH was measured by blood gas analyser,6,7 and could possibly affect antibiotic efficacy. The method we used to measure pH in the pleural empyema is less accurate than the blood gas analyser method used in these studies.11,12 Nevertheless, it is acceptable when no treatment decision is dictated by the results.

Each of the models used in the present study has its limitations. Turpentine may affect the chemical parameters of the pleural effusion,6,7 which might affect the efficacy of the antibiotic used. In the second model, the use of two pathogens causing pleural empyema is somewhat remote from normal clinical events and adds an additional confounding factor. Nevertheless, in comparison with the turpentine model, the presence of P. multocida did not affect the eradication of S. pneumoniae from the pleural empyema. The similarity in the pharmacodynamic results in the two models supports the validity of the models in the evaluation of the efficacy, pharmacokinetics and pharmacodynamics of moxifloxacin and other antibiotics in the treatment of experimental empyema.7,8

Good in vitro antibacterial activity of moxifloxacin and other fluoroquinolones against a variety of microorganisms, including S. pneumoniae, has been demonstrated previously and is little affected by pus, globulin, anaerobic conditions and low pH.13 In the present investigation, we could demonstrate that good activity is found in the animal model as well. Moreover, the lack of effect of pus and low pH on the quinolone’s antibacterial activity is in favour of the explanation that the higher effective AUIC we found was due to pockets of pus and areas of thickened pleura preventing the penetration of moxifloxacin.

These data may be valuable in further investigation of the clinical efficacy of moxifloxacin in the treatment of pneumococcal pneumonia complicated by empyema and the need to insert a pleural drain whenever pleural empyema is diagnosed.

Acknowledgements

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References


