# The impact of mechanical ventilation on the moxifloxacin treatment of experimental pneumonia caused by *Streptococcus pneumoniae*

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***Objective:*** ***Streptococcus pneumoniae*** **is a leading cause of community-acquired pneumonia and is responsible for early-onset ventilator-associated pneumonia as well. In intensive care units, community-acquired pneumonia is still associated with a mortality rate of up to 30%, especially when mechanical ventilation is required. Our objective was to study to what extent MV could influence the efficacy of moxifloxacin in a rabbit model of pneumonia.**

***Design:*** **Prospective experimental study.**

***Setting:*** **University hospital laboratory.**

***Subjects:*** **Male New Zealand White rabbits (n = 75).**

***Interventions:*** ***S. pneumoniae*** **(16089 strain; minimal inhibitory concentration for moxifloxacin = 0.125 mg/L) was instilled intrabronchially. Four hours later, a human-like moxifloxacin treatment was initiated in spontaneously breathing (SB) and mechanically ventilated (MV) animals. Untreated rabbits were used as controls. Survivors were killed 48 hrs later. Pneumonia was assessed and moxifloxacin pharmacokinetics were analyzed.**

***Measurements and Main Results:*** **Moxifloxacin treatment was associated with an improvement in survival in the SB animals (13 of 13 [100%] vs. eight of 37 [21.6%] controls). The survival rate was less influenced by treatment in MV rabbits (seven of 15 [46.1%] vs. one of eight [12.5%] controls). The lung bacterial burden was greater in MV compared with SB rabbits (5.1 ± 2.4 vs. 1.6 ± 1.4 $\log_{10}$ colony-forming units/g, respectively). Nearly all the untreated animals presented bacteremia as reflected by a positive spleen culture. No bacteremia was found in SB animals treated with moxifloxacin. In contrast, three of 13 (23.1%) moxifloxacin-treated and MV animals had positive spleen cultures. The apparent volume of distribution of moxifloxacin was lower in MV compared with SB rabbits.**

***Conclusions:*** **In our model of moxifloxacin-treated** ***S. pneumoniae*** **pneumonia, mechanical ventilation was associated with a higher mortality rate and seemed to promote bacterial growth as well as systemic spread of the infection. In addition, the volume of distribution of moxifloxacin was reduced in the presence of mechanical ventilation. Although the roles of factors such as anesthesia, paralysis, and endotracheal tube insertion could not be established, these results suggest that mechanical ventilation may impair host lung defense, rendering antibiotic therapy less effective. (Crit Care Med 2005; 33:1029–1035)**

**KEY WORDS:** ***Streptococcus pneumoniae*****; community-acquired pneumonia; ventilator-associated pneumonia; moxifloxacin; mechanical ventilation; ventilator-induced lung injury; pharmacokinetics; animal model**

*Streptococcus pneumoniae* is the main causative organism responsible for community-acquired pneumonia (CAP) worldwide. About one third of the hospitalized patients with pneumococcal CAP require intensive care unit admission (1). Despite the availability of effective antibiotic therapy and substantial improvement in the intensive care support of the patients, the mortality rate remains high when pneumonia is caused by *S. pneumoniae* (18–46%) (2–4). It is worth noting that a large proportion of such cases will require mechanical ventilation (MV) during the course of the infection. Moreover, several clinical studies have found MV to be an independent predictor of death in the setting of CAP (1, 5, 6). In addition, *S. pneumoniae* is also recovered from patients with early-onset ventilator-associated pneumonia (7).

Experimental data support the hypothesis that MV impairs lung host defense leading to a decrease in bacterial clearance (8, 9). Moreover, lung overdistention as well as the expiratory collapse of lung terminal-units caused by MV could promote pulmonary-to-systemic translocation of microorganisms and bacterial products (10–13). Taken together, these results emphasize the potency of MV in worsening the features and outcome of pneumonia.

Moreover, although MV may modify antibiotic pharmacokinetics, standard regimens are largely based on data obtained in healthy volunteers (14).

No model of antibiotic treatment of pneumonia in animals undergoing MV has been described. Therefore, the present study was designed to assess to what extent MV could influence local and systemic consequences, as well as outcome, of pneumonia caused by *S. pneumoniae* in the immunocompetent rabbit receiving a human-like treatment with moxifloxacin (15).

## MATERIALS AND METHODS

### Animals

Male New Zealand rabbits (2.5–3.0 kg) were used in the present study. The animals

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were placed in individual cages and were nourished *ad libitum* with $H_2O$ and feed, according to current recommendations by the National Institutes of Health (16). The experimental protocol was approved by the Animal Use Committee of the Dijon University. Investigators who directly care for infected animals wear a face mask to avoid any risk of human contagion.

## Preparation of the Inoculum

The bacteria used in the present study was the strain 16089 (15, 17, 18). This strain was resistant to penicillin (minimal inhibitory concentration [MIC] = 4 mg/L). The MIC of moxifloxacin determined by the standard dilution method in agar was 0.125 mg/L. Bacteria were grown in 5% $Co_2$ either in brain heart infusion broth (BioMérieux, Marcy l'Etoile, France) or on sheep blood agar plates (BioMérieux). Bacterial stocks were kept at −70°C in a 15% (vol/vol) glycerol-supplemented brain heart infusion. Before each animal experiment, the *S. pneumoniae* strain was inoculated into brain heart infusion, cultured on agar plates, and incubated for 24 hrs at 37°C in 5% $Co_2$. Then 25–30 colonies were taken and inoculated into 9 mL of brain heart infusion, incubated for 6 hrs at 37°C, and cultured on agar plates for 18 hrs at 37°C in 5% $Co_2$. This culture was diluted in physiologic saline to obtain final concentrations of 10 $\log_{10}$ colony-forming units (cfu)/mL. Concentrations were first determined by using optic density measurements in reference to a standard curve and confirmed by using successive dilution cultures.

## Mechanical Ventilation

Under general anesthesia provided by iterative intravenous injections of propofol (Rapinovet, Schering-Plough), a 3.0-mm cuffed endotracheal tube was introduced per oral into the trachea. The animal was then connected to a pressure-controlled ventilator (Drägger, Germany). MV was performed in the supine position with a continuous infusion of ketamine (Imalgene, Rhône-Poulenc, 1 mg/kg/hr) and pancuronium bromide (Pavulon, Organon-Teknika, 0.3 mg/kg/hr). Animals were hydrated by intravenous infusion of 150 mL/kg/day isotonic serum to maintain a stable hemodynamic status in the context of MV, anesthesia, and paralysis (8, 9).

Baseline ventilator settings were positive inspiratory pressure ~15 cm $H_2O$ to obtain a tidal volume of 8 mL/kg, positive end-expiratory pressure 5 cm $H_2O$, $Fio_2$ 0.5, and respiratory rate 30 breaths/min with 30% inspiratory time.

Arterial blood samples were drawn regularly for blood gas analysis as the animals underwent MV. $Fio_2$ was adjusted to maintain a $Pao_2$ >100 mm Hg, and the respiratory rate was increased if necessary to obtain a $Paco_2$ ≤45 mm Hg.

Three test rabbits were used in preliminary experiments to ascertain the safety of our ventilatory support under general anesthesia.

## Production of Experimental Pneumococcal Pneumonia in Rabbits

The development of pneumonia and the placement of the central venous catheters were performed as previously described (17, 18). Briefly, 24 hrs after jugular catheterization, bacterial pneumonia was induced by the endobronchial instillation of 10 $\log_{10}$ cfu/mL of *S. pneumoniae* diluted in 0.5 mL of saline. In animals undergoing MV, the same inoculum was administered as previously described, 60 mins after the insertion of the endotracheal tube (8).

## Moxifloxacin Treatment

After bacterial challenge, the animals were assigned to receive either moxifloxacin or saline serum. Moxifloxacin was reconstituted according to the manufacturer's instructions. The treatment was started 4–5 hrs after the lung inoculation of bacteria and lasted for 2 days. The overall dose was of 35 mg/kg/day. Antibiotics were delivered through the central venous catheter with changing infusion rates, which were calculated in an attempt to simulate the antibiotic kinetics observed in human serum when 400 mg of moxifloxacin was given intravenously once a day (maximum concentration, 4.5 mg/L; area under the concentration-time curve from 0 to 24 hrs, 40 mg·hr/L) (19). The procedure used to compensate for the faster elimination of antibiotic in small animals compared with humans was described previously (17). Briefly, from the pharmacokinetic parameters of moxifloxacin, the timed interval compensatory dose can be calculated to obtain the desired (i.e., the human) concentrations. A variable flow rate infusion with successive levels was used under the control of a programmable computer software (Softpump; World Precision Instruments, Sarasota, FL). Infusion rates were modified every 5 mins.

## Macroscopic Assessment of Pneumonia

All surviving rabbits were anesthetized and then killed by an overdose of thiopental 48 hrs after the bacterial inoculation. Autopsies were carried out directly after kill, and the lungs and spleen were removed aseptically. Animals that died early during the course of the infection (e.g., death that occurred <3 hrs after bacterial challenge) were excluded from the final analysis since death could be related to complications of the anesthesia and not to the pulmonary infection. Similarly, only animals who had received >80% of the total daily dose of moxifloxacin were included in the final analysis. Pneumonia was considered to be present if at least one congestive lesion was seen within the lung. The assessment of the severity of pneumonia was based on a macroscopic score as previously described (8, 17). Briefly, macroscopic damage evaluation took into account the worst lesion of each lobe, which was graded in five categories (from normal [1 point] to gray congestion [6 points]). The overall score was obtained by the sum of lobar score values (7–42 points). The assessment of pneumonia severity was performed in a blind manner.

## Bacteriologic Evaluation of Pneumonia

The lungs were exsanguinated. The spleen and each pulmonary lobe were weighed and homogenized in sterile serum saline. Bacteria were counted in a sample of this crude homogenate by plating successive dilutions on sheep blood agar and incubating the plates for 24 hrs at 37°C. Bacterial concentrations in each lobe and in the spleen were determined after adjusting for weight. The threshold value was 1 $\log_{10}$ cfu/g. For statistical comparisons of the difference between the pulmonary bacterial densities, culture-negative lobes were considered to contain 1 $\log_{10}$ cfu/g. For each rabbit, the mean pulmonary pneumococcal concentration was calculated according to each lobar bacterial concentration with lobar weight (e.g., mean concentration = $\Sigma$ [lobar concentration × lobar weight]/$\Sigma$ [lobar weights]). A positive *S. pneumoniae* spleen culture was considered a marker of systemic bacterial spread (20, 21).

In the treated animals and for each pulmonary lobe or spleen with residual surviving bacteria, emerging moxifloxacin-resistant mutants were detected by plating 1 mL of the crude tissue homogenate and ten-fold dilutions on sheep blood agar containing two and four times the MIC of strain. Mutants were detected if such cultures grew positive for *S. pneumoniae*.

## Pharmacokinetic (PK) Analysis

For each animal, the concentrations of antibiotics in the serum were determined on iterative blood samples, obtained through the second central catheter. Moxifloxacin concentrations were determined by a disc plate bioassay method with antibiotic medium II (Difco Laboratories) and *Escherichia coli* NIJJHC2 as the indicator organism (15). The limit of detection was 0.4 mg/L. PK analyses were per-

formed using compartmental methods (Kinetica software, Innaphase, Philadelphia, PA). A one-compartment model best fit the data on the basis of Akaike's information criterion (22). From the individual PK of each treated animal, the following variables were calculated: maximum concentration, minimum concentration, area under the concentration-time curve from 0 to 48 hrs, volume of distribution, and moxifloxacin half-life.

### Statistical Analysis

The results are expressed as the mean ± SD. Quantitative variables were compared with the Mann-Whitney U test or an analysis of variance. Percentages were compared by using the chi-square test with Yates correction or by using the Fisher's exact test, depending on the sample size. To compare relationships between quantitative values, the Spearman correlation test was used. Kaplan-Meier 48-hr survival analysis was done to describe survival in the groups stratified by type of treatment (placebo vs. moxifloxacin) and type of ventilation (mechanically ventilated vs. spontaneously breathing animals). Curves were compared by means of the log rank test. For all the tests, $p < .05$ was considered significant. All calculations were performed with Statview software (SAS Institute, Cary, NC).

## RESULTS

### Population

Three animals were used for the safety preliminary study. Two were killed after 48 hrs MV, whereas the third one was kept alive for 72 hrs. None of these animals presented macroscopic or microscopic lung abnormality.

A total of 75 rabbits were used in this study and distributed into four experimental groups as follows: spontaneously breathing infected rabbits (SB group, n = 50); mechanically ventilated infected rabbits (MV group, n = 23). In the SB group, 13 animals were treated with moxifloxacin (SB-MFX group), and 37 were used as controls (SB-C group). Similarly, 15 animals were treated in the MV group (MV-MFX group) and ten rabbits served as controls (MV-C group). As defined in the Methods section, two animals from the MV-C group and two animals from the MV-MXF group were excluded from analysis because of premature death and/or death obviously not related to pneumonia. Therefore, eight of ten animals in the MV-C group and 13 of 15 animals in the MV-MFX group were kept for the final analysis.

The mean body weight was 2.8 ± 0.2 kg, with no significant difference between groups.

### Macroscopic Findings

Gross lung examination revealed that animals from all groups had similar evidence of consolidating pneumonia in at least one lobe. Although the difference was not statistically significant, a trend toward a higher macroscopic score was noted in the animals that received MV compared with the spontaneously breathing rabbits, whether they received an antibiotic treatment or not ($p = .157$ and .090, respectively, Fig. 1).

### Bacteriologic Findings

*Bacterial Challenge.* The inoculum size was not different when SB and MV animals were compared (10.2 ± 0.2 $\log_{10}$ cfu/g vs. 10.3 ± 0.4 $\log_{10}$ cfu/g, in SB and MV animals, respectively; $p = .16$). However, it could be thought that fewer bacteria were actually seeded into the lung since the route of inoculation was not exactly the same in both groups. Therefore, the lung bacterial burden was compared between SB and MV rabbits whose death occurred within the 4 hrs following the bacterial challenge. Obviously, similar lung bacterial concentrations were achieved in both groups (9.1 ± 0.1 $\log_{10}$ cfu/g vs. 9.0 ± 0.3 $\log_{10}$ cfu/g, respectively; $p = .45$).

*Untreated Pneumonia.* High *S. pneumoniae* concentrations were obtained in control animals (Fig. 2). At the time of kill, the bacterial lung burden was found to be significantly higher in mechanically ventilated animals compared with SB rabbits (8.9 ± 0.4 $\log_{10}$ cfu/g vs. 7.1 ± 2.5 $\log_{10}$ cfu/g, respectively; $p = .009$). Based on spleen cultures, the rate of bacteremia was high since 78.4% of the SB-C group animals had positive cultures. This proportion was higher in the MV-C group (87.5%), but the difference did not reach significance ($p = .914$). Of note, the spleen bacterial concentration was strongly correlated to the lung concentrations ($r^2 = .753$, $p < .001$).

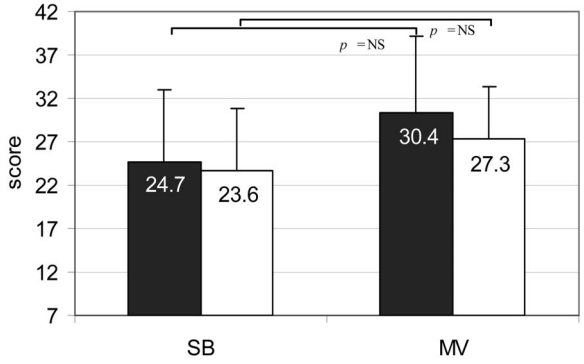


**Figure 1.** Macroscopic pneumonia severity according to the experimental group (*black bar*, control; *white bar*, moxifloxacin) in either spontaneously breathing (*SB*) or mechanically ventilated (*MV*) animals. *NS*, not significant.

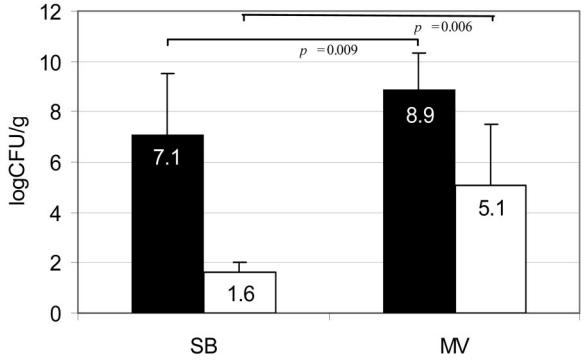


**Figure 2.** Bacterial lung burden according to the experimental group (*black bar*, control; *white bar*, moxifloxacin) in either spontaneously breathing (*SB*) or mechanically ventilated (*MV*) animals. *CFU*, colony-forming units.

*Bacterial Reduction After Treatment.* As expected, moxifloxacin was strongly effective in spontaneously breathing animals since quantitative lung cultures were sterile in 12 of 13 animals after ~48 hrs of treatment (mean bacterial lung concentration = 1.6 ± 1.4 $\log_{10}$ cfu/g, Fig. 2). No animal had positive spleen culture in this group. In contrast, lung cultures were positive for *S. pneumoniae* in 11 of the 13 MV animals that were administered moxifloxacin (5.1 ± 2.4 $\log_{10}$ cfu/g; $p = .006$, Fig. 3). In addition, a positive spleen culture was obtained in three of these animals (23.1%; $p = .220$). The spleen bacterial concentrations were also correlated to the lung concentrations in these two groups ($r^2 = .612$, $p = .027$).

If the analysis was restricted to only the rabbits that were administered the whole treatment (i.e., those with a 48-hr survival), the bacterial lung burden was also found to be significantly higher in the MV group (n = 6) than in the SB group (n = 13) (3.2 ± 2.0 $\log_{10}$ cfu/g vs. 1.6 ± 1.4 $\log_{10}$ cfu/g, respectively; $p = .04$).

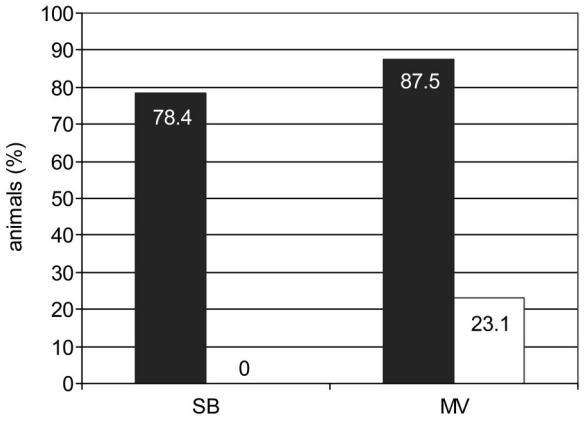


**Figure 3.** Proportions of animals with *Streptococcus pneumoniae* positive spleen culture according to the experimental group (*black bar*, control; *white bar*, moxifloxacin) in either spontaneously breathing (*SB*) or mechanically ventilated (*MV*) animals.

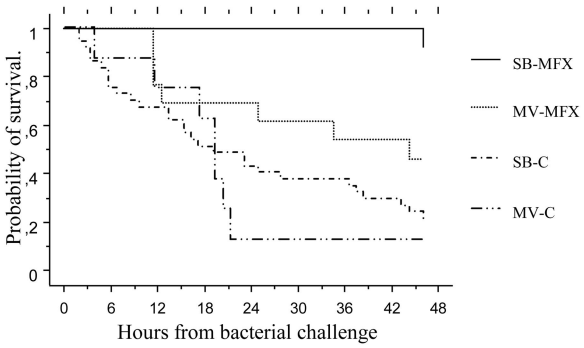


**Figure 4.** Kaplan-Meier estimated probability of survival of the rabbits after bacterial challenge with *Streptococcus pneumoniae* according to the treatment arm in either spontaneously breathing (*SB*) or mechanically ventilated (*MV*) animals (log-rank test, $p = .008$ between SB-moxifloxacin (*MFX*) and MV-MFX. *C*, control.

Of note, no rabbit harbored moxifloxacin-resistant mutant.

## Survival

In animals not treated with moxifloxacin, *S. pneumoniae* pneumonia induced similar mortality rates after 48 hrs, regardless of MV (87.5% vs. 78.4%; log-rank test, $p = .562$, Fig. 4). Although treatment with moxifloxacin was associated with a 100% survival rate among SB animals, only six of 13 MV animals (46.1%) survived ($p = .008$).

A negative spleen culture was associated with a better survival at 48 hrs when both antibiotic-treated and untreated rabbits were pooled (survival rates, 56.2 vs. 23.1%; log-rank test, $p = .003$). However, when the groups were considered separately, we failed to demonstrate a significant correlation (data not shown).

## PK Analysis

Forty-eight hours of treatment with moxifloxacin was completed in 13 of 13 animals in the SB-MFX group. Thirteen animals of 13 received ≥80% of the total daily dose in the MV-MFX group. Eight animals of 13 were given ≥30 hrs of treatment in the MV-MFX group.

Blood concentrations of moxifloxacin close to those expected in healthy human volunteers were achieved in all treated rabbits, whether they were submitted to MV or not (Table 1, Fig. 5) (23). However, there was a clear difference within groups in terms of trough concentration at 24 hrs. Trough concentrations were found to be significantly higher in the MV-MFX group than in SB-MFX animals (1.1 ± 0.4 mg/L vs. 0.6 ± 0.9 mg/L, respectively; $p = .002$, Table 1). Similarly, peak concentrations were found to be significantly higher in the MV-MFX group than in the SB-MFX group during the first 24 hrs of treatment (7.1 ± 1.9 mg/L vs. 4.3 ± 0.6 mg/L, respectively; $p < .001$). As a result, the area under the concentration-time

**Table 1.** Values of pharmacokinetic variables for moxifloxacin (MFX; equivalent to 400 mg intravenously once a day for 48 hrs) calculated in either spontaneously breathing (SB-MFX) or mechanically ventilated (MV-MFX) infected animals

| | $V_D$, L | $T_{1/2}$, hrs | $C_{max}$, mg/L | $C_{min}$H24, mg/L | $AUC_{0-48}$ mg/hr · $L^{-1}$ |
|---|---|---|---|---|---|
| SB-MFX (n = 13) | 101.4 ± 32.7 | 8.5 ± 5.3 | 4.3 ± 0.6 | 0.6 ± 0.9 | 98.8 ± 40.2 |
| MV-MFX (n = 13) | 72.7 ± 22.2 | 7.9 ± 5.6 | 7.1 ± 1.9[a] | 1.1 ± 0.4[a] | 140.5 ± 63.7[a] |

$V_D$, volume of distribution; $T_{1/2}$, half-life; $C_{max}$, peak concentration in plasma during the first 24 hrs of treatment; $C_{min}$H24, trough concentration in plasma at the 24th hr; $AUC_{0-48}$, area under the time-concentration curve.

[a]Statistical difference between the two groups.

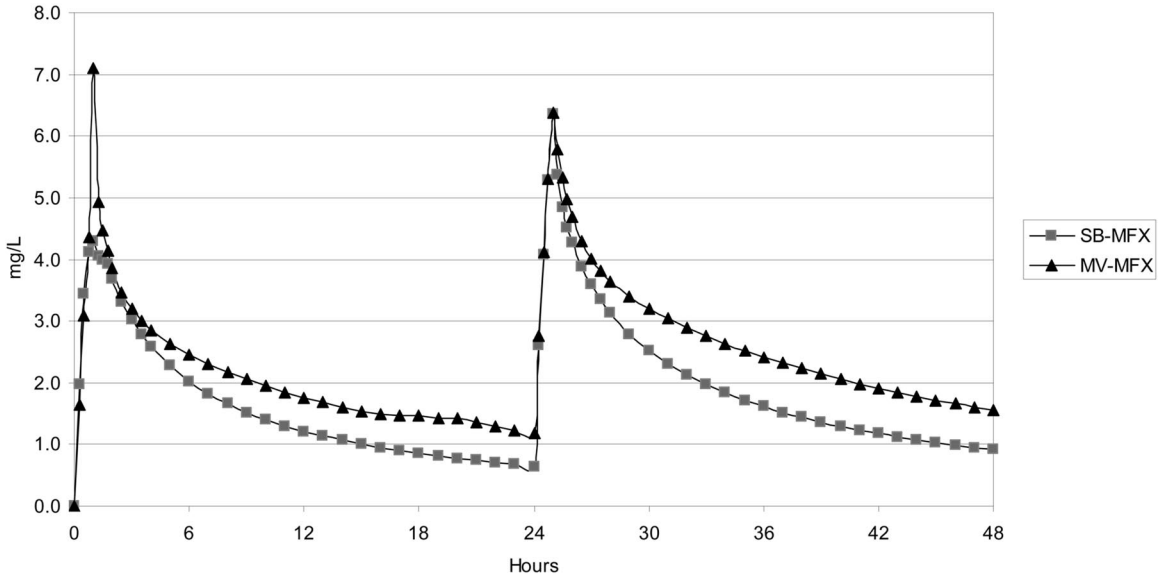


**Figure 5.** Pharmacokinetics of moxifloxacin (*MFX*; equivalent to the pharmacokinetics in humans at 400 mg once a day) in infected rabbits. *Squares*, obtained concentrations in spontaneously breathing (*SB*) rabbits; *triangles*, obtained concentrations in mechanically ventilated (*MV*) rabbits.

curve from 0 to 48 hrs was found to be greater in the mechanically ventilated animals compared with the SB group (140.5 ± 63.7 vs. 98.8 ± 40.2 mg/hr·L$^{-1}$, respectively; $p = .068$). A significant decrease of the volume of distribution of moxifloxacin could account for such a difference of concentrations between the two groups (72.7 ± 22.2 L vs. 101.4 ± 32.7 L, respectively; $p = .035$). Finally, the half-time of moxifloxacin elimination was comparable between the two groups (7.9 ± 5.6 hrs vs. 8.5 ± 5.3 hrs; $p = .809$).

## DISCUSSION

We describe here the first animal model of *S. pneumoniae* pneumonia aimed at evaluating the impact of MV on the efficacy of an antibiotic treatment. The main findings of the present study are a) when untreated animals were submitted to both MV and intrabronchial challenge with *S. pneumoniae*, lung bacterial burden was found to be higher than in SB animals; and b) whereas moxifloxacin treatment was associated with almost complete bacterial reduction and a 100% survival rate in all SB animals, mortality rate as well as lung bacterial concentrations remained high in animals subjected to MV.

Previous animal studies have shown that the use of injurious ventilation strategies (e.g., high tidal volume and zero end-expiratory pressure) leads to lung damage known as ventilator-induced lung injury (VILI) and promotes the systemic spreading of infection (10, 11, 24). More recently, several studies have suggested that MV could cause VILI despite the use of a so-called "lung-protective strategy" based usually on a ventilatory regimen associating a low tidal volume plus positive end-expiratory pressure, particularly when the lung is preinjured (8, 9, 13, 25). Our results tend to confirm such a hypothesis; however, definite conclusions cannot be drawn on the basis of our results due to the fact that we did not fully assess VILI in our model. We have, however, demonstrated this point in another model of ventilated rabbits with *Enterobacter aerogenes* pneumonia (8). Only a greater spreading of extrapulmonary infection in the MV animals—as an indirect indication of VILI—is suggestive of VILI in the present study. Our macroscopic score failed to support the presence of additional lung injury in the presence of MV. Moreover, it may be due to the lack of sensitivity since it was unable to reveal the reduction in lung damage that could be expected when an effective antibiotic therapy was administered. Another explanation for this surprising finding is that antibiotic therapy, although it led to bacterial eradication, had only a slight effect on the inflammatory lung response and its consequences, regardless of the presence of MV. We are, however, unable to provide any data regarding this point.

Caution should be exercised regarding the observed differences in outcome between the four study groups. The high mortality rate observed in the MV-MFX group may be due to a deadly combination of MV and pneumonia, but part of this mortality could also be related to the combined effects of MV, anesthesia, and paralysis. We tried to minimize these latter effects by choosing a protective ventilatory regimen. Thus, preliminary experiments showed that rabbits with healthy lungs ventilated with this regimen for ≥48 hrs could be kept alive without any sign of lung injury. Moreover, we have previously described a model of nonlethal pneumonia caused by a less virulent pathogen (i.e., *E. aerogenes*), where the rabbits were invariably alive until 48 hrs after the bacterial challenge (8). Therefore, we propose that in the present model, MV and pneumonia due to *S. pneumoniae* could act synergistically, causing mortality in the MV-MFX group. The cause of death in these animals, however, remains elusive. Our results only provide some clues as to why the outcome was significantly worse in ventilated animals. Accordingly, as a marker of bacteremia, spleen cultures remained sterile in all SB animals treated with moxifloxacin, but three of 13 ventilated animals had positive spleen cultures. Interestingly, mortality correlated with the extrapulmonary infection spread when all the animals were considered. However, the small size of our sample did not allow us to establish such a correlation in the subset of the moxifloxacin-treated animals.

Other mechanisms should be considered to explain these findings. First, the endotracheal tube bypasses natural host

*In our model of moxifloxacin-treated* Streptococcus pneumoniae *pneumonia, mechanical ventilation was associated with a higher mortality rate and seemed to promote bacterial growth as well as systemic spread of the infection.*

defenses and impairs cough and mucociliary clearance. Since the SB animals were not intubated, one cannot exclude the possibility that pneumonia was more severe in MV animals as a result of the presence of an endotracheal tube. Second, anesthesia and muscle paralysis in a prolonged supine position are associated with a modification in the diaphragm architecture and favor atelectasis and poor ventilation of dependent regions of the lung.

The new fluoroquinolone, moxifloxacin, was used in the present study because it exhibits a very low MIC against our penicillin-resistant pneumococcal strain. Indeed, moxifloxacin was highly effective *in vivo* in our immunocompetent rabbit model of pneumococcal pneumonia, as well as in humans with CAP in whom *S. pneumoniae* was the main causative agent (15, 26). However, our results do not allow us make conclusions about the effectiveness of moxifloxacin in the setting of MV-requiring pneumococcal CAP. A head-to-head comparison with state-of-the-art β-lactam treatment would be needed to address this particular point (27).

The blood PK of moxifloxacin were investigated in the present study. Interestingly, greater moxifloxacin blood concentrations were achieved in MV animals compared with SB rabbits. Similar results were obtained in mechanically ventilated critically ill patients who received ofloxacin (28). This effect was associated in humans with an increase in the half-time of elimination of the antibiotic. In our rabbit model, a reduction in the volume of distribution of moxifloxacin is more likely to account for such a difference.

It is also possible that differences in antibiotic concentrations existed in the lung, regardless of the blood data. Experimental and clinical works are sparse with conflicting results regarding this point. In animals, MV was capable of causing epithelial as well as endothelial wall disruption that could, at least theoretically, increase antibiotic lung concentrations (29). For example, vancomycin concentration in the pulmonary lining fluid was found to be dependent on the alveolar capillary membrane permeability (30). Amikacin concentrations above the MICs of common Gram-negative bacteria were achieved in lung homogenates of healthy piglets that underwent MV (31). Interestingly, these authors reported that the greatest antibiotic concentrations were obtained in the lung region where focal pneumonia developed (32). In a recent study, comparable concentrations of moxifloxacin were achieved in the plasma and in the bronchial compartment in critically ill patients with pneumonia who underwent MV (33). In contrast to these studies, it has been shown that lower antibiotic concentrations were obtained in lung homogenates of rats with multiple-system organ failure compared with healthy animals even though blood concentrations were comparable (34). In another study, insufficient concentrations of piperacillin/tazobactam were found in lung epithelial lining fluid in mechanically ventilated patients with pneumonia receiving the standard dose regimen, whereas continuous infusion of cefepime provided better results (35, 36). Therefore, antibiotic concentrations within acutely injured lungs submitted to MV are difficult to predict. In addition, the determination of antibiotic lung concentrations (e.g., antibiotic dosage made in bronchial secretion, epithelial lining fluid, or tissue homogenate) is difficult and could account for these discrepancies (37).

Since no moxifloxacin-resistant mutant was detected within the lung of rabbits from the MV-MFX group, although a significant bacterial reduction (e.g., $>2$ $\log_{10}$cfu/g) was obtained, it can be assumed that sufficient antibiotic concentrations were achieved in the lung compartment (15). We are, however, unable to provide any evidence supporting such a hypothesis since moxifloxacin lung concentrations were not measured.

There are some limitations of our model. First, results from animal studies should be taken cautiously, especially when small species are used. Indeed, it seems that small animals are more prone to VILI and bacterial translocation from the lung than larger animals (29). In addition, one cannot exclude the possibility that the differences we described in terms of outcome would have been less pronounced if a "more protective" ventilatory strategy (i.e., tidal volume of 6 instead of 8 mL/kg) had been used. Second, our analysis was made on a limited number of animals per group, especially in those with MV, which may have limited the statistical power. This may account for our failure to show any statistically significant difference between the SB-C and the MV-C groups. Third, as noted in critically ill patients, important interanimal variations in antibiotic blood concentration were observed (14). Therefore, PK analysis results should be interpreted cautiously. The mild differences in the bacterial challenge process between SB and MV animals could account for the apparent compromised ability of mechanically ventilated animals to clear out bacteria. One could consider that fewer bacteria were retained within the lung of the SB rabbits since cough was not completely abolished. However, bacterial lung concentrations were not different when only the animals that died very early after the inoculation (i.e., $<4$ hrs) were considered. Finally, one could argue that the lungs of the MV-treated rabbits exhibited higher lung bacterial concentrations when compared with the SB group because of a shorter treatment course. However, it is worth noting that similar results were obtained when only the animals that completed the 48-hr treatment were considered. Another methodological limitation is that our model does not reproduce the human CAP pathogenesis since MV was started before bacterial challenge. Therefore, it should rather be considered as an early-onset VAP model. This study design was directed by safety requirements since anesthetic drug bolus infusions were very frequently deadly in infected animals. However, our aim was not to reproduce exactly the human disease pathogenesis but rather to study the extent to which MV could influence the outcome of such an acute pulmonary infection.

## CONCLUSIONS

Our results suggest that despite an early treatment with a highly potent antibiotic against *S. pneumoniae*, immunocompetent rabbits submitted to MV were less likely to recover from pneumococcal pneumonia than animals breathing spontaneously. The observed differences in moxifloxacin PK with respect to the ventilation (e.g., SB or MV) could explain, at least partially, our findings. In ad-

dition, these suggest that increasing antibiotic dosing could be a way to optimize the management of mechanically ventilated patients with pneumonia. Finally, whether positive pressure ventilation-induced overdistention *per se* may impair local lung and/or systemic immune defense against bacterial infection remains to be determined (38). Our model seems to be well suited for further investigations that could improve our knowledge of underlying mechanisms of antibiotic-treated mechanically ventilated pneumonia.

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