Impact of early versus later fluoroquinolone treatment on the clinical, microbiological and resistance outcomes in a mouse-lung model of *Pasteurella multocida* infection.

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KEYWORDS

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ABSTRACT

The early curative uses of antimicrobial drugs such as fluoroquinolones before the onset of symptoms in veterinary medicine may be regarded as irrational antibiotic consumption. However, it should be stressed that in early curative antimicrobial treatment as in metaphylaxis, the bacterial burden at the infection site is often very low, and so the rapid eradication of the bacterial population could result.

We investigated the impact of early versus later curative administrations of 1 or 40 mg/kg of marbofloxacin on the survival of mice, the eradication of the targeted pathogen and the selection of resistant bacteria in a mouse lung infection with *Pasteurella multocida*.

In this model, for a given marbofloxacin dose, the clinical and bacteriological outcomes were better, and the selection of resistance less frequent, for the early rather than for the late treatment. Moreover, the early administration of 1 mg/kg led to better clinical and similar bacteriological (eradication and selection of resistance) outcomes than the late administration of 40 mg/kg marbofloxacin. Our results suggest that the optimal doses for the animals' cure could be lower when administered early during the time course of the infection than when administered after the disease outbreak. As the main argument against early treatments such as metaphylaxis is the possible enhancement of resistance at the gut level, further studies should assess if lower doses of antibiotic administered to all the animals of a herd could have less impact on the commensal digestive flora than higher doses only administered to animals showing clinical symptoms.

1. Introduction

The use of antimicrobial agents in veterinary medicine is regarded as a serious concern for the emergence of bacterial resistance (Aarestrup and Wegener, 1999; Phillips et al., 2004) and the antimicrobial treatments should not only aim at curing the diseased animals but also at limiting the resistance. To preserve individuals in situations at risk, a choice has to be made between two strategies: (i) an early collective treatment, also called control or metaphylaxis, in which all the animals of a group that are actually exposed to a pathogen are treated even if, at the time of treatment, only few animals show symptoms of infection or (ii) a later individual treatment in which only the animals of the group showing clinical symptoms of the disease are treated. The advantages and limitations of these two strategies need to be scientifically documented in a framework of risk-benefit analysis. The first strategy is challenged by the argument that the treatment of all the animals of a group is an overuse of antibiotics which is suspected to favour the emergence of resistant bacteria, especially in the digestive commensal flora. However, these collective treatments are launched very early after the start of the infection and consequently the bacterial load targeted by the antibiotic is supposedly lower than for the second strategy of the late treatment of animals with clinical symptoms. Therefore, the assessment of the impact of the bacterial load on the clinical and microbiological outcomes may be a key factor in making a decision between the two strategies. Indeed, previous studies have shown that the eradication of the targeted bacterial population needed lower concentrations of antibiotic (Mizunaga et al., 2005; Morrissey and George, 1999; Udekwu et al., 2009) and that the selection of resistant subpopulations of the pathogen was less frequent when a low bacterial inoculum was compared to a high inoculum (Ferran et al., 2007; Ferran et al., 2009; Kesteman et al., 2009).

In the present study, we assessed the impact of early versus late curative administration of marbofloxacin, a fluoroquinolone extensively used in veterinary medicine, on the survival of mice, the eradication of bacteria and the prevention of resistance during

the natural course of a lung infection with *Pasteurella multocida*, a bacterium responsible for pneumonia in cattle and swine.

2. Materials and methods

2.1 Bacteria and antibiotic.

A strain of *Pasteurella multocida* isolated from the trachea of a pig with clinical symptoms of a bacterial lung infection was used as the test bacteria. Marbofloxacin, a third generation fluoroquinolone, was used as the test antibiotic.

2.2 In vitro susceptibility testing.

MIC determination. The MICs were determined in triplicate according to the CLSI reference methods (CLSI, 2006).

Determination of the Mutant Prevention Concentration (MPC). The MPC was determined as previously described (Blondeau et al., 2001). Briefly, an overnight culture of the test bacteria in Mueller Hinton broth (MHB) was concentrated 100 times in 0.9% NaCl to obtain a suspension containing 10^9 CFU/mL. One hundred microliters of this suspension were then plated onto MH agar containing various concentrations of marbofloxacin obtained by successive two-fold dilutions. The MPC was the lowest marbofloxacin concentration preventing the growth of bacterial colonies after incubation for 72 hours at 37°C. Determinations were done in triplicate.

2.3 Animals.

Female Swiss mice (Charles River Laboratories, L'Arbresle, France) were used for all studies. All animal procedures were carried out in accordance with accepted human standards of animal care under the agreement number A 31909 for animal experimentation from the French Ministry of Agriculture.

Lung infection model.

Female Swiss mice were anesthetized by an intraperitoneal injection of a mixture of 0.17 mg/kg medetomidine (DomitorND, Pfizer, France) and 80 mg/kg ketamine (ChlorketamND, Vétoquinol, France). The tracheas were cannulated with a 22 G catheter without mandrel. The catheter was inserted through the glottis into the trachea until the connector was behind the incisors. The lungs were inoculated with 20 µL of a *Pasteurella multocida* suspension containing ~5.10^4 CFU/mL corresponding to a total of ~1000 CFU/lung. Most of the mice had

dyspnea, lethargy and dehydration 24 to 48 hours after the inoculation of *Pasteurella multocida*. Eighteen control mice, that were not treated, were used to assess the natural growth of *Pasteurella multocida* in the lungs. Among these mice, groups of 3 mice each were sacrificed by an intraperitoneal injection of pentobarbital sodium (DolethalND, Vetoquinol, France) 1, 5, 10, 24, 32 and 48 hours after the inoculation of bacteria and the susceptible and resistant bacteria were counted in the lungs. The pharmacodynamic assay was carried out with four groups of 14 mice each that received a single marbofloxacin dose of 1 or 40 mg/kg intraperitoneally 10 (early administration) or 32 (late administration) hours after the inoculation of the bacteria. The mice were all sacrificed 38 hours after the marbofloxacin administration. Two other groups of 14 mice that received a single dose of 1 or 40 mg/kg marbofloxacin intraperitoneally 32 hours after the inoculation (late administration) were sacrificed 16 hours after the marbofloxacin administration. There were also 28 control mice that did not receive marbofloxacin and that were sacrificed 48 hours after the inoculation. To count the susceptible and resistant bacteria, the lungs were aseptically removed and homogenized in 10 mL of 0.9% NaCl. The homogenates were centrifuged at 3000 g for 10 minutes and the pellets were resuspended in 2.5 mL of 0.9% NaCl. Ten microliters of successive 10-fold dilutions of homogenates were then plated in triplicate on MH drug-free agar plates or on MH plates supplemented with 0.128 µg/mL of marbofloxacin corresponding to the first dilution step below the MPC. The colonies were counted after 24 hours of incubation at 37°C. If the colonies were too small, incubation was continued for a further 24 hours. The lowest level of detection was 100 CFU/lung and bacteria were considered eradicated below this level.

2.4 Pharmacokinetics.

Female Swiss mice, satellites from the pharmacodynamic assay, were infected with *Pasteurella multocida* as described above and were given a single intraperitoneal dose of 20 mg/kg marbofloxacin, 10 (early administration) or 32 (late administration) hours later. Groups of 3 mice each were anesthetized by intraperitoneal injection of pentobarbital sodium (DolethalND, Vetoquinol, France) 0.25, 0.5, 1, 2, 4, 6, 8, 12, 18 or 24 hours after dosing. Blood

samples (one sample from each animal) were collected by puncture of the caudal vena cava and centrifuged at 7000 g for 10 min at 4°C. The plasma samples were stored at -20°C until assay. A simple and sensitive high performance liquid chromatography method, using ultraviolet detection at 295 nm was used to determine marbofloxacin concentrations in the plasma. Briefly, samples were extracted by solid phase extraction on a C8 100 mg cartridge. Marbofloxacin and the internal standard, ofloxacin, were separated on a reverse-phase C18 Inertsil ODS-3 column and eluted with 25 mM citrate buffer (pH=3.0) and acetonitrile in an 85:15 ratio. The standard calibration curve for marbofloxacin, using a weighted linear regression model, was linear for concentrations ranging from 0.01 to 2 µg/mL. The intra-day and inter-day precision ranged from 4.58 to 9.27% and from 4.85 to 9.46% respectively. The accuracy varied from 91 to 108%.

The marbofloxacin plasma concentrations, obtained with a single intraperitoneal marbofloxacin administration of 20 mg/kg, were analysed separately depending on the administration time (early or late), using WinNonlin version 5.2 (Pharsight Corporation, Mountain View, CA, USA). For the two groups either early or late administration, a pooling approach was used to fit the marbofloxacin concentrations to a bi-exponential model weighted by the inverse of the predicted values. The area under the concentration curve (AUC) was calculated by integrating the equation used to fit the data from zero to infinity (Gibaldi, 1982). Marbofloxacin pharmacokinetic parameters obtained from mice treated with 20 mg/kg were then used to estimate the AUC and the C_{max} (the maximal plasma concentration) values for each marbofloxacin dosing regimen (dose and administration time).

3. Results

3.1 Susceptibility studies. The MIC and the MPC of marbofloxacin for *Pasteurella multocida* were 0.016 and 0.256 µg/mL respectively. The term "resistant bacteria" in the present paper should be understood as bacteria growing in the presence of 0.128 µg/mL marbofloxacin, the first dilution step below the MPC.

3.2 Pharmacokinetics. The observed (total) and the predicted (total) plasma concentrations of marbofloxacin following single intraperitoneal doses of 20 mg/kg in mice treated 10 (early administration) or 32 (late administration) hours after the inoculation of *Pasteurella multocida* are shown in Figure 1. The AUC values obtained with the 20 mg/kg marbofloxacin dose were 16 and 54 $\mu g.h.mL^{-1}$ for the early and late administrations respectively. The corresponding clearances were 1250 $mL.kg^{-1}.h^{-1}$ and 370 $mL.kg^{-1}.h^{-1}$. Since the dose-proportionality for marbofloxacin was previously demonstrated in mice infected by a high or a low inoculum of *Escherichia coli* in the thighs (Ferran et al., 2009), we calculated the PK indices that would have been obtained for the 1 and 40 mg/kg marbofloxacin doses from the results obtained with the 20 mg/kg dose. As the binding of marbofloxacin to mice plasma proteins is less than 10 % (Ferran et al., 2009), the PK indices were determined from the total plasma concentrations. The values of AUC and C_{max} obtained after each administration time and each marbofloxacin dose are given in Table 1. Assuming dose-proportionality, the AUC and C_{max} values were proportional to the dose for a given administration time. For a given dose, the AUC values were much greater (3 -fold) for the mice treated late than for those treated early. The values of C_{max} were quite independent of the administration time.

3.3 Growth of *Pasteurella multocida* in mice

The time-course of bacteria growth in control mouse lungs is shown in Figure 2. The bacterial population inoculated in the trachea was about 1000 CFU/mouse. One hour after the inoculation, the total bacterial populations were 2.56±0.58 log_{10}CFU/lung. Ten hours after the inoculation, corresponding to the early marbofloxacin administration time in the subsequent experiments, the mice had no clinical signs of infection and the bacterial populations ranged from 5.10^3 to 2.10^4 CFU/lung and were almost 20-fold greater than at the

inoculation time. Thirty-two hours after the inoculation, corresponding to the late marbofloxacin administration time in the subsequent experiments, the bacterial population ranged from 1.10^6 to 9.10^8 CFU/lung. Hence, for the late marbofloxacin administrations, the lungs contained 25 000-fold more bacteria (from 50 to 2.10^5-fold) than for the early administrations and the clinical status of mice was worse: they showed anorexia, lethargy and dehydration. Some resistant bacteria were found 48 hours after the inoculation in one lung in a proportion of 6.10^{-4}.

3.4 Clinical and microbiological outcomes

Clinical outcome: mouse survival. The percentage of mice alive 38 hours after marbofloxacin administration for each dosing regimen (dose and administration time) are reported in Table 2. For the control group, 64% (10 died out of 28) of the mice were alive 48 hours after the inoculation. There was no mortality 38 hours after early marbofloxacin administration (i.e. 48 hours after the inoculation) included for the low dose of 1 mg/kg. Thirty-eight hours after the late administration (i.e. 70 hours after the inoculation), the high dose of 40 mg/kg marbofloxacin, only prevented the death of 10 mice out of 14. Seven mice out of 14 were dead after a late low dose of 1 mg/kg marbofloxacin.

Microbiological outcome: total bacterial populations. The percentage of mice with bacterial eradication 38 hours after marbofloxacin administration and the bacterial counts in mice without eradication are reported in Table 2. No bacteria were recovered in two mice of the control group. The early administration of the 40 mg/kg marbofloxacin dose (10 hours after the inoculation) was the only dosing regimen able to eradicate the bacteria in all the mice. The same dose of 40 mg/kg marbofloxacin administered late (32 hours after the inoculation), did not eradicate the bacteria in one mouse in addition to the 4 dead mice. The early administration of the 1 mg/kg dose (10 hours after the inoculation), even if it prevented the death of all the mice, did not eradicate the bacteria in 5 mice out of 14. For a given dose, the late administration resulted in a less frequent eradication of bacteria in the lungs than the early administration.

Resistant bacterial populations. Resistant bacteria were found in 6 infected mice in the non-treated group and in one lung per dosing regimen (dose and administration time) after marbofloxacin administration except for the early 40 mg/kg dose which had eradicated all the bacteria. The percentage of mice carrying resistant bacteria and the proportions of resistant bacteria in these mice are reported in Table 3. The proportions of resistant bacteria in the control group were always less than $1.5.10^{-3}$ whereas the proportions after an exposure to marbofloxacin were always more than $3.3.10^{-2}$ indicating selective pressure by marbofloxacin. Due to the high mortality of mice (Table 2) having received the late marbofloxacin administration and the consequent impossibility of assessing if the dead mice carried resistant bacteria or not, we decided to sacrifice two additional groups of mice 16 hours after the late administration of 1 or 40 mg/kg marbofloxacin to count susceptible and resistant bacterial populations before the death of mice. In these additional groups, resistant bacteria were assessed in all the mice except two that had received 1 mg/kg marbofloxacin and were dead at the time of sacrifice. Resistant bacteria were found in 4 lungs and in one after late administration of 1 and 40 mg/kg marbofloxacin respectively. Thus the earlier sacrifice at 16 hours after late marbofloxacin administration enabled more mice carrying resistant bacteria to be identified after the 1 mg/kg dose. The early administration of 1 mg/kg marbofloxacin and the late administration of 40 mg/kg marbofloxacin gave similar outcomes in terms of resistance since only one mouse per group had a high proportion of resistant bacteria. Therefore the dose level alone cannot explain the selection of resistance, which seemed to depend also on the marbofloxacin administration time.

4. Discussion

In veterinary medicine, to preserve the health of a herd or a flock, collective antimicrobial treatments can be administered to all the animals after the observation of symptoms in only few animals of the group. This practice is referred to as metaphylaxis which corresponds to the administration of antibiotics to animals experiencing any level of bacterial disease before overt disease (Young, 1995). According to this definition, metaphylaxis is launched after (*meta*) the start of the infectious disease whose initiating event is the disruption of the host defenses associated with a bacterial contamination or an asymptomatic bacteria carriage. In this respect, metaphylaxis should be viewed for the majority of the animals as a curative treatment occurring early in the time scale of the infection, its goal being the bacteriological cure of infected animals, which subsequently warrants the final protection (*phylaxis*) against infection outbreaks. Although these early curative uses before the onset of symptoms of a bacterial infection may be considered as overuse of antimicrobial drugs favouring the selection of resistant bacteria (Aarestrup and Wegener, 1999; Hammerum and Heuer, 2009; Lo and Cullen, 2006; Phillips et al., 2004), it must be taken into account that the pathophysiological status of the animals and the size of the bacterial load at the infection site in these early treatments (metaphylaxis) are different from the later “conventional” curative treatments launched after the outbreak of the disease. Our hypothesis was that the low bacterial load and the absence of symptoms associated with the early treatments could have more beneficial impacts for the clinical and bacteriological outcomes than the later “conventional” curative treatments. It had previously been demonstrated that antimicrobial activity may be higher for a lower bacterial inoculum (Mizunaga et al., 2005; Morrissey and George, 1999; Udekwu et al., 2009) and it was supposed that the probability of mutant bacteria resistant to fluoroquinolones being present at the time of drug administration decreased with the inoculum size. The aim of this study was to assess the clinical and microbiological outcomes of early versus later curative marbofloxacin administration to mice infected with *Pasteurella multocida*.

Previous *in vitro* and *in vivo* (Ferran et al., 2007; Ferran et al., 2009; Jumbe et al., 2003; Kesteman et al.; Mizunaga et al., 2005; Udekwu et al., 2009) studies on the influence of inoculum size on the pharmacodynamics of antimicrobial drugs did not take into account the natural course of the bacterial growth. Indeed, the different sizes of inoculum tested were directly inoculated into broth or animals, whereas in this current study, the initial inoculum of *Pasteurella multocida* inoculated into the tracheas was the same for all the mice and the initiation of marbofloxacin administration was then more or less delayed. At the early marbofloxacin administration time (10 hours after the inoculation), the mice had no clinical symptoms and this early administration could be considered as representative of the early curative use of antimicrobial drugs in the context of metaphylaxis since there was an infection (see control group) but no clinical symptoms, mimicking what is observed in veterinary practice. At the late administration time (32 hours after the inoculation), the mice were anorexic, lethargic and dehydrated and this administration was representative of later "conventional" curative treatments. As previously shown (Ferran et al., 2007; Kesteman et al.; Mizunaga et al., 2005; Morrissey and George, 1999; Udekwu et al., 2009), we found that the different inoculum sizes associated with the different administration times after bacterial inoculation had a dramatic influence on the bactericidal activity. Indeed, for a given marbofloxacin dose (1 or 40 mg/kg), the clinical outcome (survival of mice) and the eradication of bacteria were improved after early rather than late treatments. The need for a higher marbofloxacin dose to ensure the survival of mice for the late treatment was in agreement with the 20-fold higher, 50% effective dose of ciprofloxacin, for mouse survival after an inoculation of 10^9 CFU of *Staphylococcus aureus* than after the inoculation of 10^7 CFU (Mizunaga et al., 2005). In the present study, the early administration of 1 mg/kg marbofloxacin gave a higher survival rate and a similar percentage of bacterial eradication as the late administration of 40 mg/kg marbofloxacin. In the case of resistance selection at the infectious site, for a given dose, the early treatments were always associated with less selection for resistant bacteria than the late treatments. For resistance, as for bacterial

eradication, similar percentages were obtained after an early administration of 1 mg/kg and a late administration of 40 mg/kg marbofloxacin.

To assess whether the differences between outcomes after early and late treatment can be explained by differences in the exposure of bacteria to marbofloxacin, we investigated the marbofloxacin kinetics after early (10 hours after the inoculation) and late administration (32 hours after the inoculation) during the natural course of the infection. The clearance of marbofloxacin in mice receiving 20 mg/kg early after the inoculation of *Pasteurella multocida* (1250 $mL.h^{-1}.kg^{-1}$) was in accordance with the clearance in mice infected in the thighs with a low bacterial inoculum of *Escherichia coli* (1393 $mL.h^{-1}.kg^{-1}$) (Ferran et al., 2009). The evolution of the disease, as previously observed (Ferran et al., 2009; Ismail and El-Kattan, 2007; Peyrou et al., 2004) induced a lower clearance of marbofloxacin. This observation highlights the need to do pharmacokinetic studies on infected animals during drug development and also when doing experiments with rodent models in order to accurately determine the internal exposure to the antibiotic. In this study, the antibiotic clearance was three-fold lower (370 $mL.h^{-1}.kg^{-1}$) in mice given marbofloxacin late during the time course of the infection than in the mice with early administration, meaning that for the same dose, the bacteria in late treated mice had an exposure to marbofloxacin three times higher than the bacteria in mice treated early. This difference in marbofloxacin kinetics should have been an advantage for the late treatment over the early treatment for bactericidal activity and prevention of resistance. This was not the case in our experiment indicating that the three-times higher exposure associated with the same dose during the late treatment compared with the early treatment was not sufficient to compensate for the deleterious effects of the disease and/ or the inoculum size. The exposure (AUC) had to be 135-fold higher for late treatment (108 $\mu g.h.mL^{-1}$ obtained with 40 mg/kg administration) than for early treatment (0.8 $\mu g.h.mL^{-1}$ obtained after 1 mg/kg administration) to give similar outcomes. In this study, we arbitrarily decided to assess the effects of 1 and 40 mg/kg marbofloxacin and these two doses are most likely not the optimal ones for the early and late treatments of this experimental infection. However, the assessment of two doses with a 40-fold difference

enabled us to highlight that the dose may depend on the time of treatment during the course of the infection and that the optimal doses are likely to be lower in the case of early compared with late treatment.

In conclusion, our results support the hypothesis that the same dosage regimen given early during the time-course of a bacterial infection may lead to both more frequent clinical and microbiological cures and to a less frequent selection of resistant bacteria than later treatments. In addition, this study supports the view that different dosage regimens should be established to obtain required clinical and microbiological cures as well as resistance prevention depending on the type of use of an antibiotic, i.e. early or late during the course of the infection. We investigated the selection of resistance at the infection site but not on the digestive commensal flora which is the main argument against early treatments that are criticised for being an overuse of antibiotics. However, as a result of this study, we can initially reply that early lower doses gave the same microbiological and clinical outcomes as late higher doses. Indeed, even if all the animals of a herd are treated in the case of early treatment, the consumption of antibiotic per individual could be lower. By taking this element into account, further studies should now investigate at group level the impact on the overall consumption of antibiotic and on the selection of resistance, of different individual doses given to non-symptomatic animals in early “metaphylactic” curative treatments compared to diseased animals in the later “conventional” treatments.

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TABLE 1.

Values of the pharmacokinetic indices for each marbofloxacin dose and each administration time (early and late) after the infection. AUC is the area under the concentration curve and C_{max} is the maximal plasma concentration. The values were calculated from the pharmacokinetic studies carried out with 20 mg/kg marbofloxacin.

Marbofloxacin dose	1 mg/kg		40 mg/kg	
	early[a]	late[b]	early[a]	late[b]
AUC (µg/mL.h)	0.8	2.7	32	108
C_{max} (µg/mL)	0.86	1.22	34.58	48.40

[a] Marbofloxacin was administered intraperitoneally 10 hours after the inoculation.

[b] Marbofloxacin was administered intraperitoneally 32 hours after the inoculation.

TABLE 2.

Percentage of surviving mice, percentage of mice with bacterial eradication and bacterial counts in the lungs without eradication 38 hours after marbofloxacin administration for each dose and each treatment time (early and late). For the control group, the percentage was calculated 48 hours after the infection.

	Control group	1 mg/kg marbofloxacin		40 mg/kg marbofloxacin	
	48h	early[a]	late[b]	early[a]	late[b]
Percentage of surviving mice	64 % (18/28)	100 % (14/14)	50 % (7/14)	100 % (14/14)	71 % (10/14)
Percentage of mice with bacterial eradication	7 % (2/28)	64 % (9/14)	36 % (5/14)	100 % (14/14)	64 % (9/14)
Log_{10} CFU in lungs without eradication (mean±SD) [c]	6.97±1.36	4.09±1.55	4.3±2.53	-	2.53

[a] Marbofloxacin was administered intraperitoneally 10 hours after the inoculation.

[b] Marbofloxacin was administered intraperitoneally 32 hours after the inoculation.

[c] Only the lungs of mice alive and with no bacterial eradication were taken into account

TABLE 3

Percentage of lungs with resistant bacteria and proportions of resistant bacteria for each marbofloxacin dose and treatment time (early and late). The percentage was assessed 38 hours after marbofloxacin administration for the group with early administrations and the first group with late administration (late_1) and 16 hours after marbofloxacin administration for the second group with late administration (late_2). For the control group, the percentage was calculated 48 hours after the inoculation.

	Control group	1 mg/kg marbofloxacin			40 mg/kg marbofloxacin		
	48 h	early[a]	Late_1 [b]	Late_2 [c]	early[a]	Late_1 [b]	Late_2 [c]
Percentage of lungs with resistant bacteria	33% (6/18)	7% (1/14)	14% (1/7)	33% (4/12)	0% (0/13)	10% (1/10)	8% (1/13)
Proportions of resistant bacteria	$5.0x10^{-5}$ to $1.5x10^{-3}$	$6.0x10^{-1}$	$6.0x10^{-1}$	$3.3x10^{-2}$ to $1.5x10^{0}$	-	$2.0x10^{0}$	$1.5x10^{0}$

[a] Marbofloxacin was administered intraperitoneally 10 hours after the inoculation.

[b] Marbofloxacin was administered intraperitoneally 32 hours after the inoculation and mice were sacrificed 38 hours after.

[c] Marbofloxacin was administered intraperitoneally 32 hours after the inoculation and mice were sacrificed 16 hours after.

FIGURE 1: Plasma marbofloxacin concentrations versus time after administration of a single intraperitoneal dose of 20 mg/kg to mice 10 hours (early administration: ○) or 32 hours (late administration: ■) after the inoculation of *Pasteurella multocida*. Fitted concentration-time curves for the early (thin line) and the late (bold line) administration are also represented.

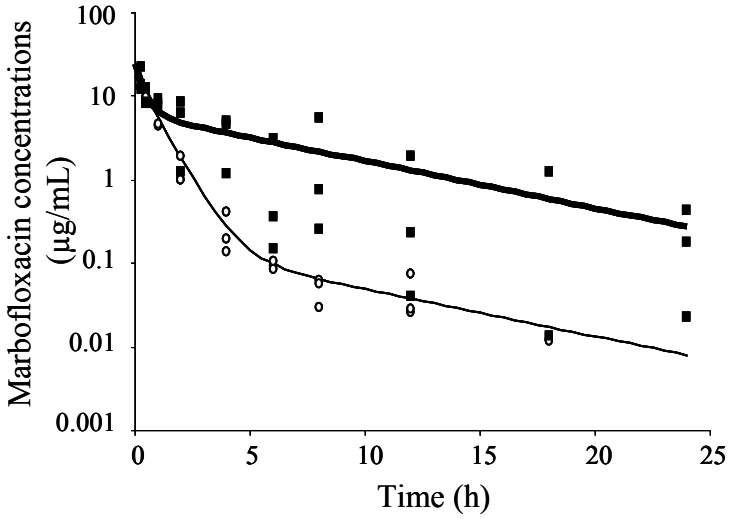

FIGURE 2: Growth of *Pasteurella multocida* in mouse lungs after inoculation into the trachea. Total bacterial populations (■) and resistant populations (□) are represented for the lungs of each mouse. Three lungs were sampled for each time point. The arrows correspond to the marbofloxacin administration times (10 hours or 32 hours after the inoculation) in the pharmacokinetic and pharmacodynamic experiments.