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Evaluation of gatifloxacin penetration into skeletal muscle and lung by microdialysis in rats

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ABSTRACT

This study aimed to investigate gatifloxacin distribution into skeletal muscle and lung interstitial fluid by microdialysis and to correlate free tissue and free plasma levels of the drug. Microdialysis recoveries were determined *in vitro* by extraction efficiency and retrodialysis at 80, 160 and 400 ng/ml resulting in 33.5 ± 1.3%, 33.1 ± 1.2%, 31.8 ± 2.7% and 31.4 ± 2.6%, 33.1 ± 2.2%, 30.6 ± 3.3%, respectively. *In vivo* recovery by retrodialysis in Wistar rats' skeletal muscle and lung were 29.1 ± 1.0% and 30.7 ± 1.4%, respectively. The recovery was constant and independent on the method or media used. Gatifloxacin tissue penetration was investigated after intravenous dosing of 6 mg/kg to Wistar rats. Free skeletal muscle, lung and plasma profiles were virtually super imposable resulting in similar area under the curve (AUC_{0-9}) of 3888 ± 734 ng h/ml, 4138 ± 1071 ng h/ml and 3805 ± 577 ng h/ml, respectively ($\alpha = 0.05$). The tissue distribution factors were 1.02 and 1.08 for muscle and lung relative to plasma. In conclusion, free plasma levels are a good surrogate for gatifloxacin active levels at the infection site.

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1. Introduction

Pharmacokinetic studies of antimicrobial drugs most often rely on plasma data. However, since most infections take place in tissues extra cellular fluids, free antimicrobial concentrations in the interstitial space at the infection site are responsible for the antibacterial effect (Marchand et al., 2005). Unless an antimicrobial is able to both sufficiently penetrate the target site and maintain an appropriate concentration in the infected tissue, it may fail to be clinically effective despite documented *in vitro* susceptibility of the involved pathogen (Joukhadar et al., 2001). Suboptimal target site concentrations of antimicrobial drugs may have important clinical implications being a potential explanation for therapeutic failure (Brunner et al., 2000; Joukhadar et al., 2001) besides triggering bacterial resistance (Hyatt et al., 1995). Although at steady-state drug levels are in equilibrium between plasma and tissue, drug penetration is not similar among different tissues and the free levels at the biophase must be known viewing to optimize antimicrobial therapy.

Community-acquired pneumonia (CAP) has an incidence of 3–5 cases per 1000 people and a mortality rate of 5–15% in hospitalized patients (Kaplan et al., 2002). Antibacterial therapy is usually indicated because of the risk of serious complications such as bacteremia and meningitis if the bacterial infection is left untreated (Liu, 2004). Despite a broad armamentarium of antimicrobials available to treat the disease, pneumonia remains the seventh leading cause of death in the United States (Minino and Smith, 2001). The management of CAP is becoming progressively complicated due to the expanding spectrum of causative organisms, the rising prevalence of resistance to antimicrobial agents and the increasing population of patients of advanced age and with comorbidities (Marrie, 1999; Rosón et al., 2001). *Streptococcus pneumoniae* is the most significant bacterial pathogen associated with community-acquired respiratory tract infections (File, 2006). Respiratory fluoroquinolones, such as gatifloxacin, are antimicrobials highly active against the pathogens most frequently implicated in CAP.

Traditionally, tissue biopsies, saliva sampling or blister fluid measurements have been used to measure drug tissue concentrations. Drug concentrations at the respiratory tract infections can be studied by assaying whole lung tissue, sputum, respiratory secretions, pleural fluid and by sampling epithelial lining fluid, especially bronchoalveolar lavage (BAL) (Brunner and Langer, 2006). BAL is currently the most often employed sampling technique for pharmacokinetic research in lung. However, calculation of the true concentration in BAL may sometimes be imprecise (Allegranzi et al., 2000), leading to overestimation of actual active concentrations in the extracellular fluid. The general limitations of BAL are

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its poor anatomical resolution and its inability to discriminate between antimicrobial active (free) and inactive (protein bound) drug. These BAL drawbacks can be overcome by *in vivo* microdialysis (Müller et al., 1995; Elmiquist and Sawchuk, 1997; Plock and Kloft, 2005).

Microdialysis is a well-established semi-invasive sampling technique that allows collection of macromolecule-free samples from complex biological matrices, including lung tissue in humans (Herkner et al., 2002; Tegeder et al., 2002; Tomaselli et al., 2003, 2004; Hutschala et al., 2005). Microdialysis principle is described in details elsewhere (Chaurasia et al., 2007). Briefly, microdialysis is based on the diffusion of compounds across a semi-permeable membrane at the tip of a probe implanted into the interstitial space fluid of the tissue of interest. The probe is constantly perfused with a physiological solution (perfusate) at a low flow rate varying between 1 and 10 μl/min. Once the probe is implanted into the tissue, substances present in the extracellular fluid are filtered by diffusion out of the extracellular space into the probe being collected sequentially over time in the dialysate (Müller et al., 2004). To obtain the compound concentrations in the interstitial space fluid from the dialysate concentrations, microdialysis probes recovery must be determined.

Tissue penetration of different quinolones has been investigated using microdialysis. The relationship between the area under the free tissue and free plasma levels (area under the curve, AUC), which indicate tissue penetration, are variable among this class of antimicrobial drugs. In general, quinolones penetrate extensively into tissues and free tissue/free plasma ratios approach to 1. The penetration ratio of ciprofloxacin was found to be 0.89 and 1.23 for subcutaneous adipose tissue and skeletal muscle, respectively (Brunner et al., 1999); for levofloxacin ratios between 0.85 in skeletal muscle (Zeitlinger et al., 2003) and 1.1 in subcutaneous adipose tissue were determined (Bellmann et al., 2004a,b); and for moxifloxacin the ratios were found to be 0.81 and 0.86 for subcutis and skeletal muscle, respectively (Müller et al., 1999a,b). On the other hand tissue penetration was less extensive for fleroxacin and norfloxacin. For fleroxacin penetration ratios of 0.34 and 0.38 were determined in skeletal muscle and subcutaneous adipose tissue, respectively (Müller et al., 1996); and for norfloxacin a 0.25 ratio was determined in rat skeletal muscle (Freddo and Dalla Costa, 2002). The protein binding of all fluoroquinolones ranges between 14 and 52% (Rodvold and Neuhauser, 2001; Bergogne-Bérézin, 2002). In this context, the free tissue penetration of each fluoroquinolone need to be investigated in order to determine the adequability of using plasma levels to optimize their dosing regimens.

The present study was conducted to investigate, by microdialysis, gatifloxacin penetration into interstitial fluids of skeletal muscle and lung of Wistar rats and to examine the relationship between free plasma and free tissue levels of the drug.

2. Materials and methods

2.1. Drugs, solvents and reagents

Gatifloxacin was provided from Bristol-Myers Squibb. Norfloxacin (internal standard) was obtained from Delaware® (Porto Alegre, Brazil). Methanol and acetonitrile, HPLC grade, as well as triethylamine and phosphoric acid 85% were purchased from Merck® (Darmstadt, Germany). Distilled water was purified by a Milli-Q system (Millipore®). Urethane was purchased from Sigma (St. Louis, USA). All others chemicals and solvents were analytical grade. Lactate Ringer's solution consisted of 149 mM NaCl, 2.46 mM $CaCl_2$ and 4.02 mM KCl.

2.2. Investigation of gatifloxacin relative recoveries

To determine the unbound fraction of gatifloxacin in the interstitial space fluid, microdialysis CMA/20 probes (10 mm membrane length, Stockholm, Sweden) with a molecular cutoff of 20 kDa were used. The Bioanalytical (Indiana, USA) microinfusion pump employed consisted of a 1020 Bee Hive controller and a 1001 Baby Bee Syringe Drive.

Both *in vitro* and *in vivo* methods were used to assess the relative recovery of the microdialysis probes. The *in vitro* recovery of gatifloxacin was determined by two different methods: extraction efficiency (EE) and retrodialysis (RD). All methods were carried out at 37 ± 1 °C. For *in vivo* evaluation only RD was employed.

2.2.1. Assessment of in vitro microdialysis

Initial experiments were performed viewing to investigate the influence of flow rate and drug concentration on microdialysis relative recovery. The influence of the flow rate was evaluated with three flow rates tested by EE and RD, using gatifloxacin at fixed concentration in lactate Ringer's solution. After selecting the flow rate, different concentrations of the drug were employed to evaluate the recovery dependency on drug concentration.

2.2.1.1. Extraction efficiency method. Blank lactate Ringer's solution was pumped through the microdialysis probes at a constant flow rate, depending on the undergoing experiment. Flows rates of 1.0, 2.0 or 3.0 μl/min were used when the influence of the flow rate on relative recovery was being investigated and a flow rate of 2.0 μl/min was used to evaluate the influence of drug concentration on recovery. The probes ($n = 3$) were placed on a glass tube filled with gatifloxacin lactate Ringer's solution. Different drug concentrations were employed in the experiments: 200 ng/ml was used when flow rates were tested and a range of concentrations (80, 160 and 400 ng/ml) were used when this factor was evaluated. A period of 1 h of equilibration of the probe in each condition was allowed before three dialysate samples were collected with 30 min intervals. The concentrations of gatifloxacin in the dialysate samples and in the solution were determined by an HPLC method previously validated. The recovery determined by the extraction efficiency method was calculated by the following equation:

$$\mathrm{EE}\ (\%) = \frac{C_{\mathrm{out}}}{C_{\mathrm{sol}}} \times 100 \qquad (1)$$

where EE (%) is the relative recovery in percentage; C_{out} the concentration in the dialysate; C_{sol} the drug concentration in the solution surrounding the probe.

2.2.1.2. Retrodialysis method. Similarly to the EE experiments, retrodialysis experiments were carried out to evaluate the influence of the flow rate (1.0, 2.0 or 3.0 μl/min) and drug concentration (80, 160 and 400 ng/ml) on probes recovery. For these experiments, in opposition to the EE previously described, the drug solution was pumped through the probes ($n = 3$) which were inserted into glass tubes containing blank lactate Ringer's solution. After 1 h equilibration, three samples were collected with 30 min intervals for each concentration and flow rate tested. The recovery in these experiments was calculated by the following equation:

$$\mathrm{RD}\ (\%) = \frac{C_{\mathrm{in}} - C_{\mathrm{out}}}{C_{\mathrm{in}}} \times 100 \qquad (2)$$

where RD (%) is the relative recovery in percentage and C_{in} is the concentration in the perfusate.

2.2.1.3. Microdialysis probe recovery over time. In order to evaluate if the microdialysis probe recovery was constant along time during

the *in vivo* investigation the following experiment was carried out. Rat plasma containing gatifloxacin (400 ng/ml) was placed into a glass tube kept at $37 \pm 1\,^{\circ}$C. Probes ($n = 3$) perfused with Ringer's solution at a rate of 2.0 μl/min were inserted into the tubes and 1 h of equilibration was allowed before sampling. Dialysate samples were continuously collected over 30 min intervals for 10 h. The recovery was determined by Eq. (1).

2.2.2. Assessment of in vivo microdialysis

The study protocols were approved by the Ethics in Research Committee of the Federal University of Rio Grande do Sul (Protocol # 2005413). Male Wistar rats (250–300 g) purchased from Fundação Estadual para Pesquisa e Produção em Saúde (FEPPS, Porto Alegre, Brazil) were used. The rats were housed under standard conditions with room temperature of $21 \pm 2\,^{\circ}$C, humidity approximately 65% and a 12-h light:12-h dark cycle. Food and water were freely available before experiments.

2.2.2.1. Retrodialysis. The animals were anesthetized with ethyl carbamate (1.25 g/kg, i.p.) and immobilized in a supine position on a dissecting board. Anesthesia was confirmed by the absence of reflexes after pinching the footpads.

For probes calibration in muscle, an incision was made in the skin of the left hind leg and a guide cannula was inserted through the skeletal muscle. The probe was placed inside the guide cannula which was removed keeping the probe in place. The probe was continuously perfused with gatifloxacin in lactate Ringer's solution (160 ng/ml) at a flow rate of 2.0 μl/min. After 1 h equilibration three samples were collected with 30 min intervals ($n = 3$). The *in vivo* recovery was determined by measuring the relative loss of the analyte diffusing from the perfusate into the extracellular fluid using Eq. (2).

For probes calibration in lung, after anesthesia, the animals were intubated by tracheotomy and artificially ventilated with room air using a rodent respirator (Harvard Apparatus, model 683) with a frequency of 62–66 min^{-1} and an air volume of 2.5 ml. The right lung was exposed through an open space cut between two ribs. A microdialysis probe was inserted into the intermediate lobe through a small incision made in the pleura. The probe was held in place with ties around the probe shaft and the lung. The lobe was carefully put back in place and the chest was superficially closed. After surgery, the procedure was the same described for probes calibration in muscle. Eq. (2) was also employed to determine the relative loss of the analyte in this tissue.

2.3. Pharmacokinetic studies

To compare gatifloxacin free tissue and total plasma levels three groups of animals ($n = 8$/group) were employed. One group was used for blood sampling; one was used to determine lung penetration and the other to determine skeletal muscle penetration of gatifloxacin. Microdialysis sampling from lung and muscle as wells as blood sampling were not harvested simultaneously from the same animals because they did not resist to the manipulations. The three groups received an intravenous bolus dose of gatifloxacin 6 mg/kg through the lateral tail vein. All animals were anesthetized as described in Section 2.2.2.1.

For blood sampling a cannula was inserted into the carotid artery. At predetermined times before (zero time) and after dosing (0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8 and 9 h), blood was withdrawn into heparinized tubes. Approximately 200 μl of blood were withdrawn in each puncture. Plasma was separated by centrifugation at $6800 \times g$, $21 \pm 1\,^{\circ}$C for 15 min and stored at $-20\,^{\circ}$C until analysis.

A microdialysis probe was implanted into skeletal muscle or lung for tissue sampling as described in Section 2.2.2.1, depending on the group investigated. The probes were perfused with lactate Ringer's solution at a flow of 2.0 μl/min for 1 h before drug administration. After drug administration, microdialysis samples were collected over 9 h, at 30 min intervals. Dialysate samples were stored at $-20\,^{\circ}$C until analysis.

The unbound concentrations in skeletal muscle and lung were calculated from the measured microdialysate concentrations and the relative recovery determined by RD *in vivo*. A non-compartmental approach was used to determine the pharmacokinetic parameters in plasma and tissues. The terminal elimination rate constant (ke) was calculated by linear regression of the natural logarithms of the last n tissue concentrations. The half-life ($t_{1/2}$) was calculated as ln 2/ke. The initial plasma concentration C_0 was determined by logarithmic back-extrapolation to $t = 0$ using the first two data points. The AUC was calculated using the trapezoidal rule up to the last data point (C_x) and adding the extrapolated terminal area, calculated as C_x/ke. The area under the first moment curve (AUMC) was calculated from a plot of C_t versus t using trapezoidal rule up to the last data point (C_x) at time t and adding the extrapolated terminal area, calculated as $C_x t_x/\text{ke} + C_x/\text{ke}^2$. The mean residence time (MRT) was calculated as AUMC/AUC. The peak concentration ($C_{\max}$) in tissue was obtained directly from the experimental data, together with the respective time to reach maximum concentration ($t_{\max}$). The tissue distribution factor (f_T) was calculated as the ratio of the unbound AUC in tissue to the unbound AUC in plasma [$\text{AUC}_{\text{tissue,free}}/f_u\text{AUC}_{\text{plasma,total}}$], where fu is the gatifloxacin unbound fraction in rat plasma.

In order to establish the relationship between plasma and free tissue levels, the profiles were fitted using the nonlinear regression program Scientist® version 2.0 (MicroMath, Salt Lake City, USA). The correlation coefficient and the model selection criterion (MSC) were used as criteria for the goodness of the resulting curve fits. The closer the correlation coefficient was to 1, the better the agreement between measured and calculated values. The higher the MSC, the more appropriate the selected model.

Total plasma levels were fitted to a two compartment open model described by the following equation:

$$C = a\,e^{-\alpha t} + b\,e^{-\beta t} \quad (3)$$

where C is the total plasma concentration; a and b are the intercepts for the distribution and elimination phases, respectively; α and β are the rate constants representing the distribution and elimination, respectively; t is time.

The pharmacokinetic constants obtained from plasma fitting were used to predict free lung and skeletal muscle concentrations using the following equation:

$$C_T = \frac{f_u(a\beta + b\alpha)}{\alpha - \beta}\left(e^{-\beta t} - e^{-\alpha t}\right) \quad (4)$$

where C_T is the free tissue level of the drug and f_u is the fraction unbound in plasma.

2.4. Gatifloxacin protein binding

Different gatifloxacin concentrations (500, 1000, 2000 and 4000 ng/ml) were used to evaluate the *in vitro* rat plasma protein binding employing microdialysis. The experiments were carried out with a pool of plasma from six animals. Microdialysis probes ($n = 3$), previously calibrated *in vitro* were inserted in plasma samples and allowed to equilibrated at $37 \pm 1\,^{\circ}$C for 1 h before sampling. The RD was carried out at a flow rate of 2.0 μl/min using lactate Ringer's solution. Three samples were collected with 30 min intervals. An aliquot for the analysis of total plasma concentration was removed before microdialysis sampling. Protein binding was determined by the ratio between the difference of total and unbound

Table 1
Pharmacokinetic parameters obtained from plasma, skeletal muscle and lung after 6 mg/kg i.v. dosing to Wistar rats ($n = 8$/group) (average ± S.D.)

Biophase	C_{max} (ng/ml)	t_{max} (h)	$t_{1/2}\ \beta$ (h)	MRT (h)	AUC_{0-9} (ng h/ml)	f_T*
Free plasma	–	–	3.3 ± 0.8	3.1 ± 0.9	3805 ± 577	–
Free skeletal muscle	1384.9 ± 290.3	0.25	4.4 ± 2.3	5.5 ± 2.8	3888 ± 734	1.02
Free lung	1792.4 ± 800.3	0.25	2.6 ± 1.0	3.6 ± 1.3	4138 ± 1071	1.08

* $f_T = \frac{AUC_{tissue,free}}{f_u AUC_{plasma,total}}$, $f_u = 0.65$.

concentration and the total concentration, after unbound concentrations were corrected by the relative recovery.

2.5. Gatifloxacin quantification in plasma and dialysate

All samples were analyzed by a validated HPLC assay using a reversed-phase C_{18} column (Shimadzu Shim-Pack CLC-ODS) and fluorescence detection (295 and 480 nm for excitation and emission wavelength, respectively) previously published (Tasso and Dalla Costa, 2007). Briefly, the mobile phase consisted of 2.5 mM phosphoric acid:methanol:acetonitrile:triethylamine (64.8:15:20:0.2, v/v/v/v) pumped at a flow rate of 1.0 μl/min. MD samples (40 μl) were directly injected into the system without any preparation. Plasma samples (100 μl) were cleaned up by solid phase extraction using a BondEluted C_{18} cartridge. For plasma analysis norfloxacin was used as internal standard (266 ng/ml). The method was linear in the range of 20–600 ng/ml. The intra- and inter-assay accuracy were up to 94.3%. The precision for the calibration standards was within 5.8%.

2.6. Statistical analysis

The differences between pharmacokinetic parameters determined for different groups or microdialysis recoveries determined by different methods *in vitro* or *in vivo* were evaluated by analysis of variance (ANOVA) or Student's *t*-test, as appropriate ($\alpha = 0.05$), employing Excel® 2003 (Microsoft®).

3. Results

3.1. Microdialysis in vitro and in vivo recoveries

The relative recoveries determined by EE were 44.6 ± 1.9%, 33.5 ± 3.9%, and 29.5 ± 1.3% and by RD were 41.2 ± 4.6%, 33.6 ± 3.2%, and 26.8 ± 2.0%, for 1.0, 2.0 and 3.0 μl/min flow rates, respectively. For the same flow rate there was no statistical difference between the recoveries determined by EE and RD.

The influence of drug concentration on recoveries was evaluated by EE and RD. The average recovery obtained for EE was 33.5 ± 1.3%, 33.1 ± 1.2% and 31.8 ± 2.7% for gatifloxacin concentrations of 80, 160 and 400 ng/ml, respectively. For RD the recoveries were 31.4 ± 2.6%, 33.1 ± 2.2% and 30.6 ± 3.3% for the same concentrations tested, respectively.

The recoveries obtained *in vivo* by RD were 29.1 ± 1.0% and 30.7 ± 1.4% for skeletal muscle and lung, respectively. For the same concentration and flow rate, the RD recovery ratios *in vitro*/*in vivo* were 1.13 and 1.07 for skeletal muscle and lung, respectively.

The time-dependency of microdialysis recovery in biological fluid determined by extraction efficiency showed that gatifloxacin recovery was time independent over a period of 10 h, proving that the experiments conducted to determine drug penetration in lung and muscle can be performed for long periods.

3.2. Protein binding

Gatifloxacin plasma protein binding was determined over the plasma concentrations range observed in animals. Gatifloxacin protein binding (35.5 ± 5.0%) was constant and independent of the concentration investigated. This average value was used for the prediction of free plasma levels based on the total concentrations determined and also for the prediction of tissue free levels.

3.3. Gatifloxacin tissue penetration

In order to investigate the tissue penetration of gatifloxacin, free drug concentration was measured in skeletal muscle and lung tissue by microdialysis after the administration of a single i.v. dose (6 mg/kg) equivalent to a dose of 400 mg administered to humans.

Pharmacokinetic parameters derived from microdialysate samples are summarized in Table 1. The peak tissue concentration (C_{max}) was not statistically different in muscle (1384.9 ± 290.3 ng/ml) and lung (1792.4 ± 800.3 ng/ml) ($p < 0.05$). The time to peak concentration (t_{max}) was 0.25 h in skeletal muscle and lung demonstrating that in rats gatifloxacin penetration into tissues occurs rapidly. The $t_{1/2}\ \beta$ was not statistically different ($p < 0.05$) across plasma, skeletal muscle and lung as well as the MRT. The area under the curve (AUC_{0-9}) across free plasma, skeletal muscle and lung tissue were very similar, resulting in tissue distribution factors close to 1, suggesting equilibrium of unbound drug concentrations among different tissues.

The total plasma profile was fitted to a two-compartment open model resulting in average a, b, α and β of 3.3 ± 0.9 μg/ml, 1.3 ± 0.5 μg/ml, 2.7 ± 1.7 h^{-1} and 0.28 ± 0.1 h^{-1}, respectively. Using these plasma hybrid constants, free tissue levels in skeletal muscle and lung were predicted using Eq. (4). Gatifloxacin profiles in plasma and tissues can be observed in Fig. 1. For both tissues, free concentrations in the interstitial space fluid were similar to the corresponding free levels in plasma at all times after dosing, demonstrating that equilibrium between free levels in plasma and tissue takes place very fast. The comparisons between predicted free concentrations in the peripheral compartment and measured free tissue concentrations by microdialysis revealed a good agreement, especially at the elimination phase. For both tissues, a good agreement between the free levels determined by microdialysis and predicted levels based on parameters calculated from plasma and protein binding, using Eq. (4), was obtained.

4. Discussion

Despite methodological and interpretative problems associated with studies of antimicrobial drug concentration in tissues employing different techniques, it is important to confirm the presence of drugs in significant concentrations at the tissues and fluids of interest for a particular infection in order to optimize dosing regimen. Considering that complete equilibrium between drug plasma and tissue concentrations cannot be taken for granted (Müller et al., 2004), free levels of a drug in a particular tissue need to be investigated. Because antimicrobials are selected to treat a particular infection based on their spectrum of activity and presumed ability to reach the infection site, the knowledge of tissue penetration is crucial for these drugs.

Free levels of a drug can be easily determined by microdialysis at different organs/tissues and the drug penetration can be compared

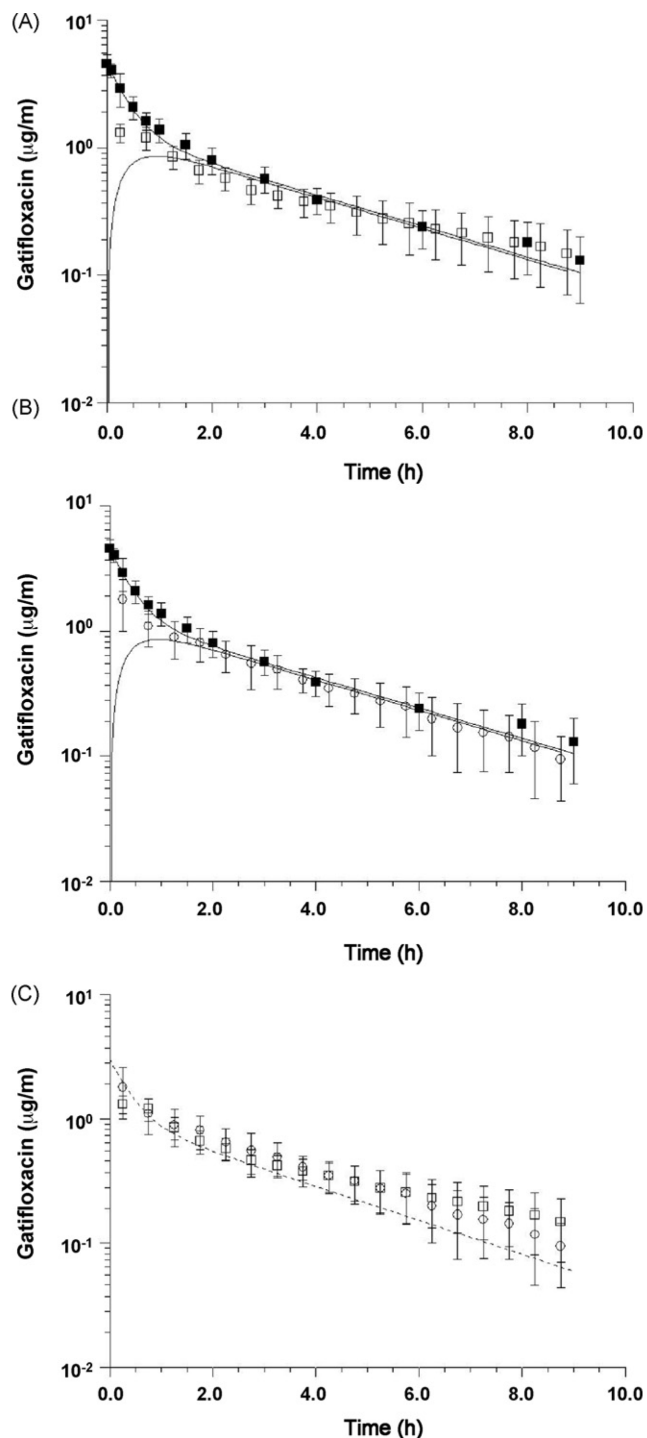

Fig. 1. Gatifloxacin profiles after 6 mg/kg i.v. bolus dosing to Wistar rats (n = 8/group). (A) Total plasma (■) and free skeletal muscle (□), (B) total plasma (■) and free lung (○). In both panels the plasma levels were fitted to a two-compartment model and the free tissue line is a prediction based on plasma data and protein binding using Eq. (4). (C) The dashed line is the calculated free plasma levels based on the determined protein binding (average ± S.D.).

among tissues as well as with plasma levels. In the present study, microdialysis was employed to measure gatifloxacin penetration into skeletal muscle and lung of Wistar rats and to correlate these with total and free plasma concentrations of the drug.

Factors related to the tissue under investigation, the conditions of the microdialysis experiment, such as flow rate and drug concentration, and the characteristics of the probes used can determine microdialysis probes recovery. Thus, in order to determine the true concentration of gatifloxacin present in skeletal muscle and lung probes recovery were determined employing different techniques.

The results showed that gatifloxacin recovery was not concentration dependent but it was inversely proportional to the flow rate. The dependency of recovery on flow rate is in agreement with previously reported data (Knaub et al., 1995; Sasongko et al., 2000).

Among the methods used to determine probes recovery, EE and RD are the most frequently employed. The gain and lost of the compound by the probe can differ depending on physico-chemical characteristics of the molecule analyzed. Since diffusion through the microdialysis membrane follows Fick's law, factors such as partition coefficient and particle size can affect drug permeability through the membrane. The flow rate used in all experiments was 2.0 μl/min which showed no statistical difference between the recovery determined by EE and RD. Independent on the method employed (EE, RD), our study showed that gatifloxacin recovery was around 30% *in vitro* as well as *in vivo*.

Drug distribution can differ among tissues and free tissue levels may also be different from those observed in plasma, leading to tissue/plasma ratios bigger or smaller than 1. In these cases it is not adequate to use plasma data to establish appropriate dosing regimens to treat tissue infections. Thus, the knowledge of tissue penetration is crucial to avoid under or overestimation of tissue concentrations. The results showed that free gatifloxacin concentration–time profile in plasma, skeletal muscle and lung were virtually superimposed, with a tissue distribution factor of approximately 1 and with pharmacokinetic parameters such as $t_{1/2}$ and AUC_{0-9} statistically similar ($\alpha = 0.05$).

Free gatifloxacin tissue levels could be predicted based on parameters determined from total plasma concentrations and protein binding in the same manner it was previously shown for some β-lactams (Derendorf, 1989; Nolting et al., 1996). Experimentally determined free muscle and lung concentrations could be well characterized as the unbound concentrations of the peripheral compartment of the model employed.

Inadequate tissue penetration of antimicrobial can lead to therapeutic failure and bacterial resistance. The results obtained in the present study suggest that no underestimation of gatifloxacin free tissue concentrations seems to occur in rats if free plasma levels are used to determine dosing regimens. Gatifloxacin demonstrated to penetrate well into healthy rats tissues. Similar findings were reported in previous microdialysis studies with other fluoroquinolones such as ciprofloxacin, moxifloxacin (Müller et al., 1999a,b), levofloxacin (Bellmann et al., 2004a,b) and gemifloxacin (Islinger et al., 2004).

It is important to consider that gatifloxacin concentrations in the interstitial fluid of normal lungs may not reflect those in the interstitial fluid of individuals with pneumonia. Although from a theoretical point of view unbound drug concentrations in plasma and interstitial fluid of peripheral tissues lacking physiological barriers such as muscle or lung should be identical at equilibrium, the relevance of using plasma antimicrobial concentrations rather than tissue concentrations has frequently been challenged, being necessary to measure the real free drug concentrations in healthy and infected tissues viewing to use them to establish relationship with total plasma concentrations. Only the confirmation that gatifloxacin free plasma/free tissue ratio is kept constant during pneumonia will assure that the dosing regimens used are appropriate to obtain effective concentrations at the site of infection.

5. Conclusions

In conclusion, microdialysis recoveries were independent of gatifloxacin concentration and around 30% *in vitro* as well as *in vivo*, independently on the method employed. Gatifloxacin showed a good diffusion through the microdialysis membrane used in this study. The results showed that free plasma levels of gatifloxacin are a good surrogate for free pulmonary and skeletal muscle levels and can be used in order to optimize dosing regimens for this quinolone.

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