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Original Article

A microdialysis study of pharmacokinetics of orally or intravenously administrated levofloxacin in lung tissues of the Streptococcus pneumoniae pneumonia rat

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Abstract: Objective: To characterize the pharmacokinetics (PK) of differently administered levofloxacin drug in the lung tissues of rats *Streptococcus pneumoniae* pneumonia. Methods: The rat model of pneumococcal pneumonia was established. Levofloxacin was administered through oral or intravenous route such that it simulated the 400 mg/d dose in patients. Microdialysis technique was used to collect the blood and lung samples simultaneously from rats with pneumonia infection. Microdialysis technique also helped to characterize the changes in the concentration of free levofloxacin. Results: 1. 20 minutes after intravenous administration, the concentration of levofloxacin reached its peak value (C_{max}) (28.08±9.88) μg/mL in lungs: the peak concentration was approximately equal to the concentration in blood. Then, the concentration decreased simultaneously in both lungs and blood. The penetrate rate (PR) of levofloxacin in lungs is 1.0111±0.21. Elimination half-life ($t_{1/2}$) is (229.9±55.6) min in blood and (232.8±74.2) min in lungs. The Area Under Curve ($AUC_{(0\text{-}inf)}$) values of drug-time curves are (4167.6±1721.6) μg·min/mL in blood and (3372.7±1086.4) μg·min/mL in lungs, respectively. The distribution coefficient (AUC_{lung}/AUC_{blood}) is (1.0394±0.85). 2. After oral administration, there were similar trends of increase and decrease in free levofloxacin concentration of blood and lungs. After 20 minutes, the average concentration in the lung tissue tends to be higher than that in the blood. The PR of levofloxacin in lungs is (1.1678±0.06). The free form of levofloxacin concentration C_{max} is (1.29±0.41) μg/mL in blood and (1.56±0.7) μg/mL in lungs. The $t_{1/2}$ is (534.4±381.6) minutes in blood and (591.4±416.1) minutes in lungs. The $AUC_{(0\text{-}inf)}$ is (920.3±473.6) min·μg/mL in blood and (1196.2±992.4) min·μg/mL in lungs, respectively. The AUC_{lung}/AUC_{blood} is (1.2188±0.56). 3. Comparing between groups, the C_{max} and AUC in both blood and lungs are significantly lower in case of oral administration. In contrast, compared with the intravenous administration, the PR in lungs is higher ($P<0.05$) when the drug is administered orally to the subjects. Conclusion: At the 400 mg/d dose, levofloxacin concentration reached the peak faster when it was administered through intravenous injection. In this case, the free drug concentration was also found to be higher in blood and lungs of rats with pneumonia. But, the drug concentration continues to be within the mutant selection window, which would potentially lead to the emergence of drug-resistant mutant bacteria. When delivered orally, the C_{max} and AUC are lower in blood and lungs; therefore, the levofloxacin antibiotic may not kill the bacteria effectively. The application of microdialysis technique in the lung tissue is safe and reliable. Moreover, the microdialysis technique can accurately characterize the pharmacokinetics of drugs in tissues. The technique of microdialysis is demarcated with higher objectivity.

Keywords: Microdialysis, levofloxacin, Streptococcus pneumoniae pneumonia, pharmacokinetics, pharmacodynamics

Introduction

Community-acquired pneumonia (CAP) is a common infectious disease, which is characterized with high mortality and morbidity [1]. Several research studies have included large-scale studies to investigate this disease. These r studies suggest that *Streptococcus pneu-*

moniae (Sp) is the most common pathogen that causes CAP [2]. In fact, the infection caused by this pathogen is so severe that most patients succumb to death, despite being hospitalized in an intensive care unit [3].

Since the 1940s, penicillin and other broad-spectrum antibiotics have been widely used. However, there has been no significant decrease in the mortality rate of patients diagnosed with CAP [4]. One of the reasons could be that at the site of infected lung tissues, the concentration of antibiotics is insufficient [5]. Furthermore, the bacteria causing CAP shows formidable drug-resistance. These two major factors are responsible for the high mortality rate of patients diagnosed with CAP.

The earlier pharmacokinetic (PK) studies of antimicrobial agents often provided insufficient information as they only reported the data associated with the plasma of subjects participating the study. However, most infections occur in the extracellular fluid of tissues. Therefore, it is more important to determine the free drug concentration in the intercellular space at the infectious site [6].

Microdialysis (Microdialysis, MD) is a novel *in vivo* technique. It can be used to collect samples in a real-time, continuous manner. MD is a unique technique as it does appreciably interfere with the normal metabolism of the subject during the process of sample collection. It is a minimally-invasive procedure, which monitors the free drug concentration in target tissues. However, very few studies have described the monitoring of lung tissues by microdialysis technique. Furthermore, the use of microdialysis technique in the study of antibiotics has been rarely reported in research studies.

In this study, we established the rat model of pneumococcal pneumonia. The rats were treated with the antibiotic levofloxacin, which was administered through the oral or intravenous routs. Samples were collected using the technique of microdialysis. The drug concentrations in blood and lungs were detected simultaneously through microdialysis. The subsequent results were combined with the *in vitro* pharmacodynamic data. In this way, the pharmacokinetics of levofloxacin delivered with different methods was characterized in the lung tissues of rats with pneumococcal pneumonia.

Materials and methods

Animals

Twelve male SD rats (SPF level, 220~250 g) were provided by the Experimental Animal Center that is affiliated to the Second Military Medical University [Experimental animal production license number: SCXK (Hu) 2007-0003; Experimental animal use license number SYXK (Hu) 2007-0003]. The maintenance of animals follows the Experimental Animal Use Protocol of the People's Republic of China. During the experiment, the following conditions were maintained: temperature 18°C-28°C; relative humidity 40%~80%; light cycle 12L/12D. Before performing the experiment, animals had free access to food and water. In this study, the experiments were approved and supervised by the Ethics Committee of the Second Military Medical University. The rats were randomly divided into 2 groups: the intravenous administration group and the oral administration group. Each group consisted of 6 rats. There was no significant difference in the total body weights of rats belonging to these two groups (238.17±11.62 g vs. 234.67±13.29 g, P>0.10).

Experimental bacteria strain

Clinical strains of *Streptococcus pneumoniae* were supplied by the Bacteria Room of Changhai Hospital's clinical laboratory. The hospital is affiliated to the Second Military Medical University. The bacteria were inoculated 1 day before the experiment on blood agar plates and cultured at 37°C for 24 h. Bacteria were diluted with sterilized physiological saline. In this way, we prepared a suspension solution having a concentration of 4 McFarland units (determined by the turbidimetric method). The quality-control strain was the standard *Streptococcus pneumoniae* (ATCC49616, Bao Mi Ke Biotech, Shanghai, China).

Equipment

Micro pump (CMA402), fraction collector (CMA 820), 1 mL syringes, vascular probes (CMA/20PAES, dialysis membrane length: 10 mm, molecular weight cut-off 100 kD, diameter: 0.5 mm), and Lung probes (CMA/15PAES, dialysis membrane length 5 mm, molecular weight cut-off 100 kD, diameter 0.5 mm) were purchased from CMA Microdialysis (Sweden). LC-2010AHT

HPLC was purchased from Shimadzu (Japan). Kromasil C-18 column (150 × 4.6 mm, 5 μm) and ALC-V8S small animal respiration machine were supplied by Shanghai Alcott Biotech (China).

Chemicals and reagents

Levofloxacin Hydrochloride standards (National Institute for the Control of Pharmaceutical and Biological Products, purity 99.8%); Levofloxacin Hydrochloride bulk drugs (Wuhan Hengzhong Biochemical Co., China. Purity 99%. Catalog Number 20090901); Sodium pentobarbital (Sigma-Aldrich, purity ≥98%, Catalog Number P3761); ACD buffer (Sodium citrate 22 mg/ml, citrate 8 mg/ml and glucose 24.5 mg/ml; Shanghai Transfusion Technology, Catalog number 09081621B); methanol (TEDIA, chromatographic pure); ultrapure water; Triethylamine and phosphoric acid (Analytical grade).

Establishment of pneumonia model in rats

Rats were anesthetized with an intraperitoneal injection of 3% sodium pentobarbital. Subsequently, they were fixed in an overhead position on a 37°C warm pad. Successful anesthesia was confirmed by no reflex when footpads were squeezed. Neck skin was sterilized. The trachea was exposed through a longitudinal incision. Thereafter, a puncture was made between the tracheal rings. 0.2 Ml of *Streptococcus pneumoniae* suspension (about 2.4×10^8 CFU/rat) was injected into these rats. In order to ensure a uniform distribution of bacterial solution in the lungs, the rats were kept in a standing position for 20 s. After 5 days, the same injection procedure was repeated. The pneumonia model was successfully established 9 days after inoculation.

Animal experiment methods

After the rats were anesthetized, tracheal intubation was performed. The breath was maintained using a small animal ventilator (frequency 80 beats/min, tidal volume 2.5 ml). In order to expose the right lobes, an inclined incision was made on the right side of the chest. The 5^{th} rib was also partially broken for this purpose. The right middle lobe was suspended with the help of non-toothed forceps. The microdialysis probe was implanted in the hilar direction of the lung tissue. After fixing the probe, the lobe was carefully brought back and the chest was closed [7]. Vascular probe was implanted in the inferior vena cava through the left femoral vein.

Determination of probe recovery rate

Reverse dialysis method was used. After successful probe implantation, the lung probe was perfused with Ringer buffer, while the vascular probe was perfused with ACD buffer. Thus, any chances of coagulation at the dialysis membrane of the probes were minimized. After a stabilizing period of 2 hours, the Ringer and ACD buffers were replaced with buffers containing 1 μg/mL standard levofloxacin hydrochloride. The lungs and vessels were perfused, while maintaining a flow rate of 2 μl/min. Thus, the solutions were collected every 10 minutes. The drug concentrations in the perfusion solution (C_{perf}) and dialysis solution (C_{dial}) were determined using the analytical technique of high pressure liquid chromatography (HPLC). The *in vivo* recovery rate of probes was calculated using the formula: $R_{dial} = (C_{perf}-C_{dial})/C_{perf}$. The measured *in vivo* drug concentration C_u was calculated using the formula $C_u = C_m/R_{dial}$. Here, the dialysis concentration C_m was determined by continuous *in vivo* monitoring.

Pharmacokinetic analysis

Based on the ratio of body surface area in rats and human-beings, we calculated the dosage of levofloxacin antibiotic that would be suitable for rats (42 mg/kg).In this way, we could simulate the levofloxacin dosage of 400 mg/d, which is recommended for adult patients. The dosage specifications of levofloxacin antibiotic have been fixed after taking into account the instructions of the Chinese State Food and Drug Administration. The levofloxacin solution was diluted to 1 mL. The levofloxacin solution was intravenously injected through the tail vein in rats. On the other hand, gastric tube was used to orally administer the antibiotic in rats. The process of administering the injection was completed within 15 seconds. Thereafter, dialysis samples were immediately collected from these injected rats.

During the first 240 minutes of the experiment, the samples were collected at regular time intervals of 10 minutes. Thereafter, the samples were collected every 20 minutes. At this stage, the process of sample collection was performed for 360 minutes. Meanwhile, changes in drug concentration in blood and lungs

were simultaneously monitored for 360 minutes.

The pharmacodynamic data were obtained with the help of Phoenix Win Nonlin (Pharsight Corporation, Version 6.1, 2009) software. Non-compartment models were used for this purpose.

Based on the measured value, area under the curve was calculated using the formula: $AUC = AUC_{(0-t)} + AUC_{(t-inf)} = AUC_{(0-t)} + C_{last}/\lambda_Z$.

Area under the first moment was calculated using the following formula: $AUMC = AUMC_{last} + (t_{last} \times C_{last}/\lambda_Z) + C_{last}/(\lambda_Z)^2$.

Here, λ_Z is the slope of the tail index section on the log drug concentration-time curve. C_{last} and t_{last} are the drug concentrations of the terminal observed value. Mean residence time MRT = AUMC/AUC.

Drug elimination half-life: $t_{1/2} = \ln(2)/\lambda_z$.

Blood and lung drug distribution coefficient AUC_{lung}/AUC_{blood}.

Penetration rate of levofloxacin in lungs $PR = C_{lung}/C_{blood}$.

Statistical analysis

Statistical analysis of data was performed with the help of SPSS13.0 statistics software. Measurement data were expressed in terms of mean ± SD. Pharmacokinetic parameters that required statistical analysis were subjected to homogeneity of variance analysis. This analysis helped us to confirm the normal distribution of the pharmacokinetic data. Thereafter, the data were analyzed with two-sample *t* test ($P<0.05$ indicates statistical significance).

Results

Levofloxacin chromatographic conditions

Specificity: Under current experimental conditions, there was no endogenous interfering substances affect the measurement. The peak shape of levofloxacin concentration and resolution were good. The retention time was 3.58 minutes.

Linearity and scope

Levofloxacin hydrochloride standard was accurately weighed and dissolved in ultrapure water to prepare stock solution. The concentration of the stock solution was 1 mg/mL. In order to prepare a series of standard solutions in triplicates, the stock solution was subjected to further dilutions. The concentrations of these diluted standard solutions were 0.0304, 0.0608, 0.2435, 0.487, 1.95, 7.8125, 31.25, 62.5, and 125 μg/mL, respectively. All these standard solutions were prepared by diluting the different aliquots of stock solution. 10 μL of each standard solution was tested by HPLC, and the peak area was recorded. The plot of levofloxacin peak area (Y) vs. concentration (X) was subjected to linear regression in order to obtain the standard curve $Y = 54680.4X-12610.8$ ($r^2 = 0.9998$). According to the standard curve, the linearity of levofloxacin concentration is good for dialysis solutions where the concentration lies between 0.0304~125 μg/mL.

The precision and accuracy

The standard solutions of low, medium, and high concentration of levofloxacin were prepared by diluting the stock solution appropriately. Thus, the respective concentrations of the standard solutions were 0.0608, 1.95, and 62.5 μg/mL. The standard solutions were continuously tested six times in one day. Thereafter, the actual concentration was determined using the standard curve of the day. Within-day precision, the Relative Standard Deviation (RSD) values of the measured concentrations were 0.62%, 6.45%, and 3.18%, respectively. With the help of this method, measured concentrations were determined for three consecutive days. Thereafter, the RSD values were calculated after taking into account the day-to-day precision. These RSD values were found to be 0.87%, 6.28% and 5.55%, respectively. It is interesting to note that the RSD values of the within-day precision and day-to-day precision were less than 15.0%. In other words, this method meets the requisite requirements of methodological precision. Solutions of different concentrations were prepared, 5 replicates for each concentration, and analyzed with HPLC. The signals were used to determine the measured values with standard curve regression equation.

Relative recovery was calculated using the following formula:

Relative recovery = (measured value/added concentration) × 100%.

Table 1. The within-day and day-to-day precision and accuracy of the measurements of biological samples

Added concentration (μg/mL)	Found concentration (μg/mL)	RSD (%)	Recovery (%)
Inter-day			
0.0608	0.0577±0.0036	0.62	94.8±5.94
1.95	1.86±0.12	6.45	95.2±5.93
62.5	61.38±1.95	3.18	98.2±3.11
Intra-day			
0.0608	0.0596±0.0052	0.87	98.06±8.62
1.95	1.91±0.12	6.28	100.97±5.89
62.5	60.58±3.36	5.55	99.9±6.69

According to the results, the accuracy of this method varied between 94.8% and 101%. The results were in the range of 100±15%. Therefore, this method is suitable for measuring biological samples (**Table 1**).

Pharmacokinetic results

Before performing intravenous administration of drugs, probe recovery was conducted in rats using the method of *in vivo* reverse dialysis. According to estimated reports, the recovery was (36±12) % in blood and (28±11) % in lungs. After completing the procedure of sample collection, all animals were checked by thoracotomy. No pulmonary bleeding or significant pleural effusion was found in rats that participated in this study. The probe was fixed inside the lung tissues. These tissues were dissected in presence of the probe. Thereafter, the dissected lung tissues were fixed with formalin and subjected to paraffin section. Based on this technique, we could confirm that probe implantation sites consisted of lung parenchyma.

Drug-time curve

As shown in **Figure 1**, the log curves displayed the concentrations of levofloxacin hydrochloride at different sample collection points in blood and lungs. The standard of levofloxacin hydrochloride was administered through oral or intravenous routes. Twenty minutes after intravenous administration, the concentration of free levofloxacin in lungs was measured. The results showed that this concentration increased significantly within a short time interval of twenty minutes. In fact, the results suggested that the concentration of free levofloxacin in lungs was approximately equal to its concentration in blood. Thereafter, levofloxacin concentrations decreased simultaneously in blood and lungs. The drug-time curves depicting free levofloxacin concentrations in lungs and bloods were so similar that they almost overlapped each other. On the other hand, after oral administration of levofloxacin, the concentrations of this free drug in increased in blood and lungs. Furthermore, the free drug concentrations in blood and lungs also showed a subsequent simultaneous decrease. It is important to mention that the concentration of free drug in lungs was slightly greater than that in blood. In order to perform statistical analysis of the data, the concentrations at different time points in blood and lungs were analyzed with the help of *t*-test. All the *P* value exceeded the threshold value of 0.05.

The drug-time curves that were plotted using the time points from different drug delivery methods could be clearly distinguished as each of them had their own individual characteristics. There was no intersection between the curves depicting free drug concentrations in blood and lungs. The curves were quite distinct for both: intravenous and oral routes of administration. While comparing intravenous and oral modes of administration, it was found that the concentrations of free drug are higher with intravenous administration in both blood and lungs

Comparison of pharmacokinetic parameters of blood

After intravenous and oral administration of levofloxacin hydrochloride, the PK values in blood were determined (**Table 2**). After intravenous or oral administration of the drug, the C_{max} values in blood were 40.39±21.83 μg/mL and 1.29±0.41 μg/mL, P = 0.010. The $AUC_{0\text{-inf}}$ values were 4167.6±1721.6 min·μg/mL and 920.3±473.6 min·μg/mL, P = 0.009. The difference is statistically significant.

Comparison of pharmacokinetic parameters in lung tissues

After administering the levofloxacin hydrochloride through intravenous or oral route, the PK values in lungs were determined (**Table 3**). After

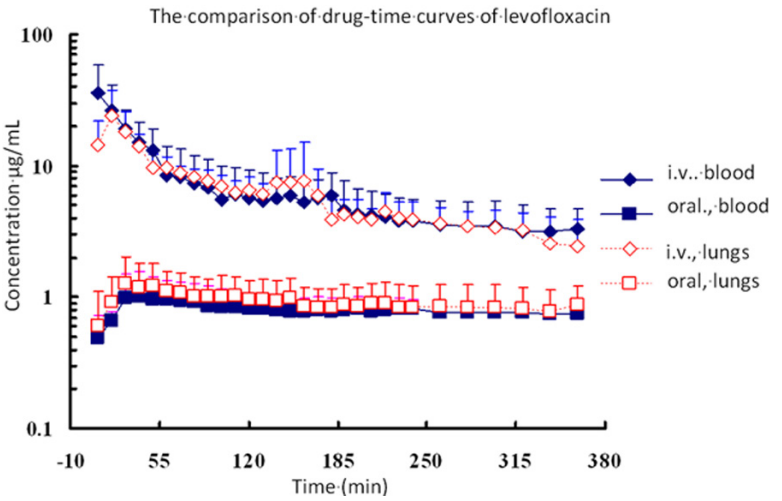

Figure 1. The curves of levofloxacin concentrations at different sample collection sites. Two different drug delivery methods were employed in this process. The data were represented as mean ± SD.

Table 2. The comparison of PK parameters of levofloxacin hydrochloride in blood with intravenous and oral administration

AUC_{lungs}/AUC_{blood}	Intravenous (n = 6)	Oral (n = 6)	*P* value
t1/2β (min)	229.9±55.6	534.4±381.6	0.116
tmax (min)	10	95±98.15	0.089
C_{max} (μg/mL)	40.39±21.83	1.29±0.41	0.010
AUC_{0-t} (min·μg/mL)	3035.3±1229.3	311.8±36.6	0.003
AUC_{0-inf} (min·μg/mL)	4167.6±1721.6	920.3±473.6	0.009
MRT (min)	269.6±93.4	814.9±533.8	0.057

The data were presented as mean ± sd. C_{max} denotes the peak concentrations of drug in tissues, whereas tmax represents the time elapsed in reaching the peak value. AUC_{0-inf} is the total area under the curve and AUC_{0-t} represents the cut-off area that extends to the terminal observation point. MRT is the mean retention time of drug molecules *in vivo*.

Table 3. The comparison of PK parameters of levofloxacin hydrochloride in lungs with i.v. and oral administration

AUC_{lungs}/AUC_{blood}	Intravenous (n = 6)	Oral (n = 6)	*P* value
t1/2β (min)	232.8±74.2	591.4±416.1	0.096
tmax (min)	20	70±56.57	0.084
C_{max} (μg/mL)	28.08±9.88	1.56±0.7	0.001
AUC_{0-t} (min·μg/mL)	2361.2±550.6	351.4±124.6	0.0002
AUC_{0-inf} (min·μg/mL)	3372.7±1086.4	1196.2±992.4	0.017
MRT (min)	287.7±107.3	883.6±599.6	0.062
AUC_{lung}/AUC_{blood}	1.0394±0.85	1.2188±0.56	0.729
PR value (C_{lung}/C_{blood})	1.0111±0.21	1.1678±0.06	0.0002

intravenous or oral administration of levofloxacin, the C_{max} values in blood were found to be 28.08±9.88 μg/mL and 1.56±0.7 μg/mL, respectively (P = 0.010). Furthermore, the AUC_{0-inf} values were 3372.7±1086.4 min·μg/mL and 1196.2±992.4 min·μg/mL, respectively (P = 0.017, the difference is statistically significant). Similar distribution coefficients were associated with the two drug delivery methods. After oral administration, the penetration rate of levofloxacin was higher in lungs, 1.1678±0.06 vs. 1.0111±0.21 (P = 0.0002, the difference is statistically significant).

Discussion

In the past few decades, the strains of *Streptococcus pneumoniae* have become quite resistant to penicillin and other antibiotics. In fact, the resistance rate of these strains has been growing rapidly. This trend has been observed in many parts of the world, especially in recent decades. In other words, their drug resistance has become a problem of worldwide concern. The SENTRY monitoring results of 1998 to 2004 suggested a gradual decrease in the sensitivity of *Streptococcus pneumonia species to* β-lactam and macrolide antimicrobial drugs. *Streptococcus pneumonia species* were isolated from the respiratory tracts of patients visiting the community healthcare centers. The SENTRY monitoring operation was performed in 123 centers, which were spread across 39 countries [8].

In 2007, the Infectious Diseases Society of America and American Thoracic Society (IDSA/ATS) revised the guidelines of CAP treatment [9]. According to revised guidelines, respiratory quinolones should be used initially in empirical therapy used for treating CAP clinically. This approach is

recommended in instances where *Streptococcus pneumoniae* show high resistance to macrolide antibiotics. While using levofloxacin antibiotic in the treatment of pneumococcal pneumonia, the drug concentration in blood should be maintained above the mutant prevention concentration (MPC). Thus, one can ensure successful treatment also prevent the emergence of drug-resistant mutants.

In 1999, Zhao and Drlica et al. in the US first proposed the hypotheses of mutant prevention concentration (MPC) and mutant selection window (MSW), which provided novel insights to the practice of clinical optimization of antibiotics. Their hypotheses took into account the drug dosing regiments of antibiotics and restricted the selective amplification of drug-resistant mutants. Some scientists have suggested that the MPC-related PK/PD parameters are more appropriate parameters in this issue. In addition, AUC_{24}/MPC and C_{max}/MPC could be considered as the ideal PK/PD parameters to predict the extent of drug resistance. AUC_{24}/MIC = 20 h can be used as the lower limit of MSW, while AUC_{24}/MPC = 25 h can be used as the upper limit of MSW *in vivo*.

Several controlled clinical studies have proved the bacteriological and clinical efficacy of levofloxacin antibiotic in CAP treatment. In addition, sequential therapy can reduce the hospitalization time and maintain the efficacy of the treatment. Sequential therapy is provided to patients through oral and intravenous routes of administration. In earlier times, the PK was determined with the help of samples procured from the plasma or bronchoalveolar lavage of healthy subjects. However, the free drug concentration in target tissues is the most important PK parameter. Moreover, the AUC of antibiotic drugs administered to patients with bacterial infections is not equal to the AUC of antibiotic drugs administered to healthy subjects.

When lungs are infected by the bacteria, pathogens are primarily situated in interstitial lung extracellular fluid, because it is the main target of antimicrobial agents. Using microdialysis technique, we investigated the PK characteristics of levofloxacin drug in infected rat lungs. For this investigation, the extracellular drug concentration was determined in a real-time and continuous manner.

According to previous research studies in China, the MIC_{90} and MPC_{90} of *Streptococcus pneumonia* have concentrations of 1 mg/L and 4 mg/L, respectively [12]. When single dose of levofloxacin was given to simulate the 400 mg/d dose in human, the C_{max} values in blood and lungs was significantly higher than the MIC_{90} and MPC_{90} of *Streptococcus pneumonia*. Thus, we found that better bacterial removal effects could be achieved through this approach. On the other hand, according to the MSW theory, the $AUC_{(0\text{-}inf)}/MIC_{90}$ is 69.46 h and $AUC_{(0\text{-}inf)}/MPC_{90}$ is 17.37 h in blood; while $AUC_{(0\text{-}inf)}/MIC_{90}$ is 56.21 h and $AUC_{(0\text{-}inf)}/MPC_{90}$ is 14.05 h in lungs. All the values lie between the upper and lower limits of MSW. Thus, these PK parameters can possibly induce drug-resistant Streptococcus.

When the same dose of levofloxacin was administered orally, C_{max} and $AUC_{0\text{-}inf}$ values were found to be significantly lower than the intravenous group ($P<0.05$). The C_{max} values are 1.29±0.41 μg/mL and 1.56±0.7 μg/mL, respectively. Thus, they are slightly higher than MIC_{90} but lower than MPC_{90}. In lung tissues, the $AUC_{(0\text{-}inf)}/MIC_{90}$ is only 19.94 h. Thus, it is close to the lower limit of MSW. Furthermore, the $AUC_{(0\text{-}inf)}/MIC_{90}$ is 15.34 h: a comparatively lower time period. Therefore, while simulating the 400 mg dose of human through oral administration, levofloxacin may not be able to attain the effective tissue drug concentration in lung tissues of infected rats. In addition, because AUC/MIC_{90} value is closer to the lower limit of MSW, the possibility of induced drug-resistance in Streptococcus cannot be ruled out. But the results also indicate that, with both oral and intravenous administration, levofloxacin has good tissue distribution and penetration in rats with pneumonia, and they are significantly higher than those in normal human lungs [13, 14].

Furthermore, after oral administration, the PR reached 1.1678±0.06, which is higher than the PR attained through intravenous administration. A possible explanation could be that in inflammatory situation the local blood vessels in the infected tissues get dilated and blood flow gets accelerated. Meanwhile, with congestion and edema in lung tissues, the permeability of blood vessel walls increases, promoting inflammatory cells entering the tissue space. Thus, the penetration of levofloxacin into lung tissues gets enhanced.

According to the findings of this study, oral administration may not be the best choice

under current dosage conditions of levofloxacin in the initial stages. Therefore, intravenous administration should be chosen. However, the dose should be increased to prevent the emergence of drug-resistant bacteria strains. Nevertheless, further studies should include human subjects as there are significant biological differences between experimental animals and human beings.

Conclusion

In this study, rats were infected with *Streptococcus pneumoniae*. Thereafter, they were treated with the antibiotic levofloxacin. It should be noted that levofloxacin exhibited strong penetration and tissue distribution coefficient in lung tissues. This powerful penetration of the antibiotic was found in both oral and intravenous modes of administration. When the infected rats were intravenously administered with the dose equivalent to 400 mg in humans, levofloxacin reached optimum concentration in blood and lungs. Thus, the antibiotic could successfully eliminate the *Streptococcus pneumonia* from the infected rats. But, the concentration is still within the range of mutant selection window. In other words, we cannot rule out the possibility of induced emergence of drug-resistant mutant strain. After oral administration, the concentration and AUC value of levofloxacin drug decreased significantly in blood and lungs. The value is lower than the effective therapeutic concentration. It is necessary to adjust the dose and deeply investigated the PK/PD of levofloxacin. Microdialysis technique is a reliable and safe procedure that can be applied to lung tissues. In addition, the data generated by this approach provide important information, which can be used in the study of Pharmacokinetics of antibiotic drugs.

Disclosure of conflict of interest

None.

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