Full Length Research Paper

A simple and novel method for preparing the taste masking levofloxacin microsphere suspension

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A taste masking levofloxacin resinate microsphere suspension was prepared. At first, levofloxacin-resinates were prepared by both bath and column methods in HCl solution (0.01 mol/L) before they were used as cores to prepare the suspension. The drug loading amount in the drug resinates made by bath method and column method was 33.2 ± 0.8 and 45.7 ± 0.6% (n = 6), respectively. The average diameter of the drug resinates was 120 μm. The scanning electron microscopy (SEM) results showed that there was no drug crystallization on the surface of the drug resinates. The in vitro drug release results showed that the drug resinates did not release levofloxacin in water, and the drug released faster with the increase of ion concentration in the dissolution mediums. The taste masking evaluation of the suspension was carried out among 6 volunteers; taste evaluation revealed that the suspension masked the bitter taste of the drug completely. Lastly, in vivo pharmacokinetics evaluation was carried out in beagle dogs. The in vivo pharmacokinetics test showed that the suspension and reference (levofloxacin tablets) had no significant differences for $C_{\text{max}}$ and $AUC_{0-36h}$, but indicated a significant difference for $T_{\text{max}}$. The $T_{\text{max}}$ decreased from 1.83 h (reference) to 1 h (test), indicating a faster release property in vivo.

Key words: Levofloxacin, ion exchange resin, suspension, in vivo, taste masking.

INTRODUCTION

Levofloxacin is a third-generation fluoroquinolone that is active against a broad spectrum of pathogens. It is the ofloxacin’s laevo-isomer, and it is used to treat a broad array of Gram positive and Gram negative bacterial infections (Deng et al., 2011; Drozak et al., 2008). Since its application in clinical practice in 1994, levofloxacin usage has increased steadily in hospital and community settings (David et al., 2012). Levofloxacin has a favorable pharmacokinetic profile and shows excellent efficacy in both clinical studies and therapy for various infectious diseases (Leandro et al., 2011). Levofloxacin is well absorbed from the gastrointestinal tract after oral administration and can be given without regard to food. The absolute bioavailability of levofloxacin tablets is nearly 100%, peak plasma concentrations of levofloxacin usually occur 1 to 2 h after oral dosing under fasted conditions. The favorable pharmacokinetic profile of levofloxacin makes the oral and intravenous routes of administration to be considered interchangeable, which will greatly benefit the patients. In particular, the oral suspension will greatly benefit the patients with deglutition difficulty and it will be easy to administer tailored doses specific for individual cases. Nevertheless, most of the fluoroquinolones suspensions taste very bitter, which will limit the oral routes of administration. Therefore, it is essential to develop a suitable taste masking oral fluoroquinolones suspension. Taste masking technologies rely on the prevention of interaction between the drug molecule and the oral mucosal surface. By creating a physical barrier around each particle, the drug substance can be prevented from going into the solution and interacting directly with taste receptors. Recently, several studies...
have been carried out to mask the fluoroquinolones bitter taste in suspensions (Gao et al., 2006; Sohi et al., 2004; Kikuchi et al., 2007; Kim and Choi, 2004). These studies have made some obvious progress; however, the conventional techniques have individual shortcomings. Firstly, the taste masking methods with flavours, sweeteners and amino acids cannot thoroughly prevent the bitter taste. Secondly, the key excipient lecithin in the method with lipophilic vehicles is too expensive. Thirdly, the technology in the method of coating with hydrophilic vehicles is too complex, with the possibility of coating corruption which will cause the bitter taste (Jackson et al., 2001). All the aforementioned shortcomings make the conventional techniques not ideal for the industrial manufacture of the taste masking fluoroquinolones suspension products. In contrast, the ion exchange resins can overcome the aforementioned shortcomings through their low cost, simple manufacture process, and the thorough prevention of drug release in oral cavity. The drug resinates suspension has been proved successful for the taste masking liquid drug delivery (Sambhaji et al., 2004).

Ion exchange resins are high molecular weight polyelectrolytes, which can exchange mobile ions of similar charges with the surrounding medium. Recently, they have been applied to drug delivery systems (Boonsong et al., 2008; Hideki et al., 2001; Thomas et al., 2008), primarily controlled release systems in the liquid form, and taste masking. The drug resinates suspension using ion exchange resin as the drug carrier has been proved successful for the taste masking liquid drug delivery (Geetha et al., 2004). However, this drug resinates suspension still has its shortcoming, presently, it can only load ionic type drug and there have been few studies on the application of slightly soluble drug. In this article, we novelty ionized the \(-\text{NH}_2\) group in the slightly soluble drug-levofloxacin and we carried out detailed research on the application of ion exchange resin in the slightly soluble drug suspension that has a bitter taste (Cheng et al., 2002).

The purpose of this study was to prepare a taste masking levofloxacin-resinate suspension. Levofloxacin-resinates were prepared by both bath and column methods before it was used as the core to prepare the suspension. The drug loading, particle size, scanning electron microscopy (SEM), and in vitro drug release were investigated (Seong and Kinam, 2008). The taste masking evaluation of the drug resinates was carried out among 6 volunteers. Finally, the in vivo pharmacokinetics evaluation of the levofloxacin-resinate suspension was carried out in beagle dogs.

**MATERIALS AND METHODS**

Levofloxacin was obtained from Dong Ya Pharmaceutical Factory, Taizhou, China. The cation exchange resins Amberlite® IRP69 (sodium polystyrene sulfonate) was obtained from Rohm and Haas Company, Philadelphia, PA, USA. Microcrystalline cellulose (Avicel RC591) was obtained from FMC Corporation, Brussels, Belgium. Tween80 and methyl-p-hydroxybenzoate were obtained from Sigma-Aldrich Corporation, St. Louis, MO, USA. All reagents were of analytical grades.

**Preparation of levofloxacin-resinates with the bath method**

2 g of ion exchange resins were suspended in 200 ml HCl solution (0.01 mol/L) containing 2 g of levofloxacin with magnetic stirring at 30°C for 3 h. The levofloxacin-resinates were then washed off the physical absorbed levofloxacin on the resinates with deionized water and were dried at 40°C (Halder et al., 2005; Akkaramongkolporn and Ngawhirunpat, 2003).

**Preparation of levofloxacin-resinates with the column method**

For the column process, a glass column (size: 1.0 × 25 cm, bed volume: 25 ml) was used (Jeong and Park, 2008). 2 g of ion exchange resins were slurried with water and transferred to the glass column equipped with a coarse fritted glass disk at the bottom. To stabilize the packing, the ion exchange resins were backwashed with water using a peristaltic pump before 200 ml 10 mg/ml drug in the HCl solution (0.01 mol/L) was pumped upward at a rate of 50 ml/h. The levofloxacin-resinates were then washed off the physical absorbed levofloxacin on resinates with deionized water and were dried at 40°C.

**Determination of drug loading in the drug resinates**

The levofloxacin loading amount in the drug resinates was determined by suspending 150 mg levofloxacin-resinates in 100 ml HCl solution (0.25 mol/L) magnetically stirred for 6 h at 60°C. The solution was then filtered, and the amount of levofloxacin in the filtrate was determined using ultraviolet (UV) spectroscopy at 293 nm.

**Particle size analysis by dynamic light scattering**

The mean particle size of the drug resinates was measured by using a laser light scattering particle size analyzer (LS230, Beckman Coulter, Miami, FL, USA) according to user’s manual.

**Morphology of the drug resinates**

The surface morphology of the drug resinates was examined by the SEM (Jeol JSM-6400, Tokyo, Japan). Samples were gold sputtering coated (BAL-TEC SCD004, Liechtenstein) for 165 s at 15 mA in an atmosphere of argon.

**Preparation of levofloxacin-resinates suspension**

Drug resinates (equivalent to 200 mg of levofloxacin) were mixed with 1 g Avicel RC591, 45 mg Tween80 and 45 mg methyl-p-hydroxybenzoate in the 100 ml deionized water. After the suspension was prepared, it was gently shaken at appropriate intervals during the storage of 1 week.

**In vitro drug release**

In vitro drug release was investigated following the USP paddle
Assessment of the bitter taste of the levofloxacin (Bitterness threshold)

The bitter taste threshold value of levofloxacin was determined based on the bitter taste recognized by six volunteers (3 females and 3 males). A series of levofloxacin aqueous solutions were prepared at different concentrations as standard solutions, that is, 10, 20, 30, 40, and 50 μg/ml, respectively. The test was performed as follows: 1 ml of each standard solution was placed on the center of the tongue, it was retained in the mouth for 30 s, and then the mouth was thoroughly rinsed with distilled water (Pisal et al., 2004; Liu et al., 2001). The threshold value was correspondingly selected from the different levofloxacin concentrations as the lowest concentration that had a bitter taste.

Taste masking evaluation

Taste evaluation of drug resinates was performed by 6 volunteers in the age group of 19 to 22 years. The study protocol was explained and written consent was obtained from the volunteers. 100 mg of levofloxacin powder and the drug resinates equivalent to 100 mg levofloxacin were separately held in the mouth for 30 s by each volunteer. Bitterness levels were recorded both instantly and after 30 s. The bitterness level was recorded against pure drug by using a numerical scale. The numerical scale was used with the following values: 0 = tasteless, 1 = acceptable bitterness, 2 = slight bitterness, 3 = moderately bitterness and 4 = strong bitterness (Rashmi and Rajesh, 2010; Liu et al., 2000).

In vivo pharmacokinetics study

The in vivo pharmacokinetics evaluation was performed by a crossover treatment in six healthy beagle dogs (weighting 15 ± 5 kg) with a 7-day washout period. The beagle dogs were fasted overnight for at least 12 h with free access to water. Food was not allowed until 4 h after the oral administration (Varaporn and Greepol, 2008). The levofloxacin-resinates suspension was used as test preparation. The conventional levofloxacin tablets (Beijing Zhizhu Pharmaceutical factory, 20110503, 100 mg levofloxacin/tablet, Beijing, China) were used as reference preparation. Both preparations contained a dose of 200 mg levofloxacin. The test and reference preparations were orally administered to the beagle dogs along with 50 ml water, respectively. All studies were conducted in accordance with the Principles of Laboratory Animal Care (NIH publication no.85-23, revised in 1985), and were approved by the Department of Laboratory Animal Research at Shen zhen Flying Century Biotech Co., Ltd. 5 ml of blood samples were taken at the following time points: 0; 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 h after oral administration. The blood samples were then centrifuged at 4000 rpm for 10 min. Plasma was harvested and stored at -20°C until further use.

Analysis of levofloxacin in plasma samples

The amount of levofloxacin in plasma was determined by the following procedure. Plasma samples (400 μl) were mixed with 50 μl 20 μg/ml gatifloxin (internal standard) solution and 10% HClO₄ aqueous solution (200 μl) and the mixture vortexed for 5 min. After that, each sample was centrifuged at 10000 rpm for 10 min. The supernatant was then analyzed by using a high performance liquid chromatography (HPLC) system which was equipped with a LC-10AT HPLC pump (Shimadzu, Kyoto, Japan), a SPD-10AVP UV Spectrometer detector (Shimadzu, Kyoto, Japan), and a Diamonsil C₁₈ column (200 x 4.6 mm, 5 μm particle size; Dima Co., Ltd., Orlando, FL, USA) (Tasso et al., 2008; Tasso and Costa, 2007). The sample injection volume was 20 μl and the UV detection wavelength was 293 nm. Analysis was performed at 35°C by using a mobile phase (water:acetonitrile:triethylamine, 82:19.5: v/v). The mobile phase was adjusted to pH 3 with phosphoric acid at a flow rate of 1.0 ml/min. The analysis method was validated according to the established international guidelines and requirements (Validation of analytical Methods: Definitions and Terminiology, ICH Topic Q2A, and Validation of Analytical Procedure: Methodology, ICH Topic Q2B). No interfering peaks were detected at the retention times of levofloxacin and gatifloxin (Figure 1). A linear correlation (r > 0.999) was obtained from the ratio of the peak areas of levofloxacin to that of the internal standard in the levofloxacin concentration range of 0.2 to 20 μg/ml. The limit of quantification (10×background noise) was 0.8 ng. The precision and accuracy of the method were evaluated at concentrations of 20, 10, and 0.2 μg/ml. The precision of the method was assessed on the basis of the coefficient of variation among quality control samples and the accuracy was calculated as the bias (%) of these and 0.66 to 2.10% at all concentration, respectively. The bias (%) of the intra- and inter-day accuracy was 0.54 to 1.54% and 0.61 to 1.62%. No decrease in the content of quality control samples was observed in the freezer.

Pharmacokinetics study

The pharmacokinetic parameters including the maximum plasma concentration (Cmax) and the time of the maximum plasma concentration (Tmax) were observed from the plasma concentration to time curve. The areas under the serum concentration-time curve (AUC₀₋₃₀) were calculated by the trapezoidal method. The results from the two preparations were analyzed with an SPSS statistical package by analyzing the variance to assess any significant (P < 0.05) differences.

Statistics

Data was obtained at least in triplicate and expressed as the mean ± standard deviation (SD). Statistical differences were determined by the student’s two-tailed t test. Differences are considered statistically significant at P < 0.05.

RESULTS AND DISCUSSION

Levofoxacin-resinates preparation

Because the –NH₂ group in levofloxacin could be ionized by H⁺ in the acid solution, the solubilities of levofloxacin increased significantly in the acidic solution. However, the H⁺ in acid medium could also competes the active sites with levofloxacin and result in a decrease in the drug
Figure 1. HPLC chromatograms of levofloxacin in plasma samples. A, blank plasma; B, blank plasma added with levofloxacin and gatifloxacin; C, plasma sample obtained from a dog added with gatifloxacin. Peaks: 1, levofloxacin; 2, Gatifloxacin.

Figure 2. The size distribution.

loading amount. In this study, HCl at an optimized concentration of 0.01 mol/L was used for the levofloxacin-resinates preparation.

According to the Stoke theory, the particle size would enhance the physical stability of the suspension made of drug resinates for the clinical application.

Particle size analysis

The size distribution of the drug resinates is as shown in Figure 2. The average particle diameter was 120 μm.

Morphology of the drug resinates

The scanning electron micrograph of the drug resinates is as shown in Figure 3. The results showed that there was
no crystallization of drug on the surface. This observation can also be used to explain the taste masking ability of the resinates.

**Drug loading in drug resinates**

The UV results showed that the drug loading amount in drug resinates made by the bath and the column methods was $33.2 \pm 0.8$ and $45.7 \pm 0.6\%$ ($n = 6$), respectively. There are two well-known methods to prepare the drug resinates: bath method and column method. Bath method is easy to operate, but by using ion exchange, the $H^+$ will be exchanged from the ion exchange resins into the solution, it will compete the ion exchangeable position with the drug and hence, the drug loading will be decreased. In contrast, the $H^+$ exchanged from the ion exchange resin will be washed away without competing the ion exchangeable positions with the drug in the column method. In this study, the drug loading of the column method is much higher than the bath method. Therefore, we chose the column method to prepare the drug resinates. The following results are all from the drug resinates made by column method.

**In vitro drug release**

The *in vitro* drug release results are as shown in Figure 4, the results showed that the drug resinates did not release levofloxacin in water, and with the increase of ion concentration in the dissolution mediums, the drug released
faster. These results can be explained by the fact that the drug release process was mediated by the ion exchange functionality from the resinate. The results showed that levofloxacin combined with the ion exchange resins with chemical bonds instead of a physical mixture. Because there are almost no ions in humans' oral cavity, the drug will not be released from the drug resinate. Therefore, people will not feel the bitter taste of drug resinate in the oral cavity. The dissolution media used should simulate the digest fluid in gastrointestinal tract. 0.15 mol/L HCl was used as the dissolution medium since the drug release process is an ion exchange process from the drug resinate and the ion concentration of electrolytes in the digest fluid is approximately 0.15 mol/L. The results showed that 90% of the drug was released within 60 min, due to the fact that the resins have an immense superficial area for ion exchange with the digest fluid.

### The bitterness threshold of levofloxacin

The bitterness threshold of levofloxacin recognized by the volunteers was between 20 and 30 μg/ml. It was found that the threshold value of levofloxacin was found to be 30 μg/ml from the majority of the volunteers.

### Taste evaluation by panel method

The results of the taste masking test are listed in Table 1. The mean score of the drug resinate had significant difference with drug powder, the score of drug resinate was much less than that of the drug powder. The results indicated that the drug resinate sufficiently alleviated the bitterness of levofloxacin. Because the drug resinate is insoluble, they have virtually no taste, so that even very bitter drugs lose their taste when converted into drug resinate. In addition, the drug will not be released in the mouth as there are almost no ions in the oral cavity, so the patient will not taste the drug when it is swallowed. When the drug resinate contacted with the gastrointestinal fluids, there will be enough ions for the ion exchange process, and the drug will be released from the drug resinate quickly and completely and will eventually be absorbed in the usual way.

### In vivo pharmacokinetics study

Figure 5 shows the levofloxacin plasma concentration-time profiles of the test and the reference preparation. Table 2 summarizes the pharmacokinetic parameters and the relative bioavailability of the test preparation, obtained by using the following equation:

\[
Fr = \frac{AUC_{(0-36 \text{ h})} (\text{test})}{AUC_{(0-36 \text{ h})} (\text{reference})}
\]

The comparison of the parameters between the two preparations showed no significant differences for \( C_{\text{max}} \) and \( AUC_{0-36 \text{ h}} \), but indicated a significant difference for \( T_{\text{max}} \). The \( T_{\text{max}} \) decreased from 1.83 (reference) to 1 h (test), indicating a faster release property in vivo, it due to these fact that the drug resinate is a polydispersity delivery system, according to the tablet, drug resinate have much higher surface area for drug release, so it has faster drug release in vivo. In the in vitro release, the drug resinate can completely release more than 90% levofloxacin within 60 min in the stimulated digest fluids, so the drug absorption in vivo also speeded up. The relative bioavailability of the test preparation also showed no significant differences from the reference. The results showed that the taste masking levofloxacin-resinate suspension was bioequivalent with reference.

### Conclusions

In the present study, a novel taste masking levofloxacin-resinate suspension was prepared. The drug and the resins were complexed by the chemical bond. The levofloxacin-resinate suspension showed an ion concentration dependent release profile and a complete taste masking. The in vivo pharmacokinetics study also showed a faster release profile with the bioequivalence

### Table 1. Taste masking evaluation.

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Drug powder Instant score</th>
<th>Drug powder 30 s score</th>
<th>Drug resinate Instant score</th>
<th>Drug resinate 30 s score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>1</td>
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<td>1</td>
<td>1</td>
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<tr>
<td>6</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Sum</td>
<td>22</td>
<td>20</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>ANOVA</td>
<td>-</td>
<td>-</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>
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0
2
4
6
8
10
12
14
16
0
6
12
18
24
30
36
Time (h)
Drug concentration (mg/L)
Reference
Test

Figure 5. Plasma concentration-time curve of levofloxacin following oral administration of reference and test preparation. Each point represents the mean ± SD (n = 6).

Table 2. Pharmacokinetic parameters of levofloxacin after a single dose of testing preparation and reference preparation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference preparation</th>
<th>Test preparation</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{1/2}[ke]$ (h)</td>
<td>8.88 ± 1.56</td>
<td>9.12 ± 1.29</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>$k_e$ (h$^{-1}$)</td>
<td>0.078 ± 0.019</td>
<td>0.076 ± 0.012</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>$T_{max}$ (h)</td>
<td>1.83 ± 0.258</td>
<td>1.00 ± 0.274</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>$C_{max}$ (μg/ml)</td>
<td>13.52 ± 1.66</td>
<td>9.69 ± 1.09</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>$AUC_{0-36h}$ (μg/h/ml)</td>
<td>177.26 ± 23.16</td>
<td>179.97 ± 25.68</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>$AUC_{0-∞}$ (μg/h/ml)</td>
<td>190.11 ± 21.23</td>
<td>193.03 ± 22.07</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Relative bioavailability</td>
<td>-</td>
<td>101.53 ± 3.14%</td>
<td>ANOVA</td>
</tr>
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