Therapy Scheme and Pharmacokinetics of Injecting Florfenicol in Contagious Caprine Pleuropneumonia Goats

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Abstract: A study was conducted to provide a theoretical basis and clinical dosage regimen for using Florfenicol (FFC) to prevent Contagious Caprine Pleuropneumonia (CCPP) in goats. Twelve healthy goats were divided into two groups: Group A designated as the healthy, non-inoculated, control group and Group B designated as the CCPP treatment group. All goats were injected with a single dose of FFC at 20 mg/kg body weight intramuscularly (IM). Plasma concentrations of FFC and minimal inhibitory concentrations (MIC) of FFC on Mycoplasma sp. that causes CCPP were determined. Pharmacokinetic parameters were calculated by the 3P97 computer program. The results indicated that there was no obvious difference of pharmacokinetics of FFC in healthy goats and CCPP goats. The characteristics of pharmacokinetics of the IM injecting of FFC in all goats demonstrated fast absorption, broad distribution, effective concentration and slow elimination. Main parameters of the pharmacokinetics were as follows: $\text{T}^{1/2}_{\text{Ka}} = 0.41 \pm 0.25$ h, $\text{T}^{1/2}_{\text{Ke}} = 7.41 \pm 3.66$ h, $\text{T}_{\text{peak}} = 1.57 \pm 0.57$ h, $\text{C}_{\text{max}} = 4.64 \pm 2.15$ mg/l, $\text{AUC} = 54.38 \pm 20.65$ mg/h/l. The MIC was $0.18 \pm 0.07 \mu$g/ml. A FFC clinical therapy scheme for goats diagnosed with CCPP is as follows: administration cycle ($\tau$) = 24.21 h, first dose ($X_0$) = 21.20 mg/kg, maintaining dose ($X$) = 20 mg/kg, times of administration ($n$) when the $f_{ss}$ reaches 99.9% = 2.38

Keyword: goats, Contagious Caprine Pleuropneumonia, Florfenicol, pharmacokinetics

Introduction

Florfenicol (FFC), a broad spectrum antibiotic used for animal health and a fluorinated analog of thiamphenicol (Plumb, 2002), has been successfully used for the prevention and cure of many diseases in cattle, pigs, chickens, sheep, dogs, cats and aquaculture. Although the United States Federal Drug Administration (FDA) approves its use in cattle alone, it has been useful in other species of animals. Currently, there are limited reports and observations of the use of FFC in goats.

In recent years, CCPP, a respiratory tract and an World Organization for Animal Health (OIE)-listed disease (Guangyin, C. 2008), has resulted in serious harm and losses on goat production in the world (Gelagay et. al., 2007; Ozdemir et al., 2005; Daihua and Gangyi, 2005), especially in Asia and Africa. This disease infects only goats and exacerbates lesions isolated to the respiratory tract (Smith et al., 1994). It can be easily diagnosed clinically with the following signs: fever, cough, a painful respiration with grunting, purulent nasal discharge, and reluctance to move around (Smith et al., 1994).

The first reported occurrence of the disease in the world was in 1873. In China, the disease first occurred in 1947 (Daihua, W. and Gangyi Xu. 2005). In 1976, MacOwan and Minette (1976) confirmed that the etiological agent of CCPP was a Mycoplasma sp.. Transmission of the disease occurs mainly by aerosolization during cohabitation (Smith et al., 1994). In 2003, there were 14 countries with incidences of CCPP reported by OIE, including India, Iran and Libya, with high mortality rate. During the recent years, the disease has resulted in great economic losses because of the introduction of large numbers of goats for the development of intensive goat production. Incidence of the disease reached above 80% with a mortality rate of 40% (Derong et al., 2000; Changjiang et al., 2002; Juan et al., 2003) in China. Most of the goats infected with CCPP were below 3 years of age.
FFC has been widely used in preventing many diseases of animals due to good absorption, wide antibacterial spectrum, widespread body distribution, high rate of bioavailability, and without latent side effects of aplastic anemia. The objective of the study was to provide a theoretical basis and clinical dosage regimen for using FFC to prevent CCPP in goats.

**Materials and Methods**

**Drugs and Equipment**

A FFC standard product (99.6%) was dissolved with chromatogram level acetonitrile to a FFC standard solution with a concentration of 1000 mg/l. FFC crude drug (98.3%) was dissolved to a 20% FFC injection with N, N-2-methyl acetamide and propanediol. Chloramphenicol (99.9%) was dissolved with acetonitrile and dual-distilled water at 500 mg/l and used as a CM reference solution. All above the drugs were provided by Censorship Station of Veterinary Drugs of the Sichuan Province, and preserved at -4ºC until they were used.

The standard strain of mycoplasma of CCPP (PG3) was separated and provided by Prevention and Veterinary Laboratory of Sichuan Agricultural University. A CCPP (PG3) diagnostic kit and Hayflick’s mycoplasma medium were provided by Lanzhou Veterinary Institute, Academy of Agricultural Sciences of China.

Main instruments for testing included SHIMADZU LC-2010C HT High-Pressure Liquid Chromatograph (Made in Japan), D-37520 Type High Speed Centrifuge (Made in Germany), SW-CJ-IF Purity Bench and SK 250 L/H Ultrasonic Washing Unit (Made in China). The chromatographic column was SHIMADZU VP-ODS C18 (250×4.6 mm, 5µm). Chromatographic conditions were as follows: UV detection wavelength (λ) at 223nm, mobile phase of acetonitrile-water (30:70), flow velocity at 1.0 ml.min⁻¹, column temperature at 30ºC, and sampling size of 20 µl.

**Animals Treatments and Plasma Administration**

Fourteen crossbred goats (Boer × Chengdu Ma, a known local breed in Sichuan Province) at 6 months of age with a body weight of 20 kg were provided by the Goat Breeding Center of Sichuan Agricultural University. All goats were fed according to conventional methods and had access of fresh drinking water. They were observed and quarantined for 2 weeks prior to the initiation of the treatment. Physical examinations and clinical serologic testing confirmed the goats to be healthy prior to experimentation.

Among the 14 goats, 12 were randomly selected and allotted into two groups: Group A was designated as the healthy, non-inoculated, control group; and Group B was designated as the CCPP treatment group. The goats were each injected intramuscularly with a single dose of FFC at the level of 20 mg/kg body weight. The remaining two goats were used for animal regression tests, CCPP model identification, dissection and histological examinations.

All goats were fasted for 24 hours prior to the administration of FFC. Blood samples (5 ml) for each goat were collected at 0.083, 0.167, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12, 24 hour after the administration. Anticoagulation of the blood sample was accomplished using heparin sodium. The plasma was separated by centrifugation at 4,000 x g for 10 minutes and preserved at -20ºC.

**CCPP Pathological Model**

The standard strain of CCPP was inoculated in Hayflick’s liquid medium and was cultivated at 37ºC until the mycoplasma concentration reached above 107 Color Change Unit (CCU) /ml. It was then diluted to 104 CCU/ml. Each goat in Group B was inoculated with 4 ml of the diluted liquid via trachea.

Upon completion of the CCPP model and according to the standard method of OIE on separating mycoplasma (Jones, 1992; Lixin et. al., 2002), the purulent nasal discharge of goats in Group B were collected. The samples collected were used for the separation of mycoplasma strain of CCPP, identification of character of the strain, biochemistry identity test, growth inhibition test, animal regression test, pathological dissection, and histological examination.

**Standard Curve Administration and MIC Test**

The FFC standard solution of 1000 mg/l was made into a series of six standard solution concentrations ranging from 0.5 to 400 µg/ml. For each series, 20.0 µl of solution was mixed with 1.0 ml of blank plasma, 10.0 µl of CM and 1.0 ml of phosphate buffered saline (PBS) at a pH of 7.0 by vortex motion. A quantity of 4.0 ml of ethyl acetate was then added to the mixed solution and again mixed by vortex motion for extraction. The upper strata of the solutions were collected after centrifuging at 4,000 x g for 15 minutes. The extracting process was repeated according to the above method by ethyl acetate. The series of six solutions were contained in 6 individual test tubes and dried using nitrogen gas and a 60ºC water bath. Each series was then preserved at -20ºC.

The remnants following the extraction of the upper strata of the solutions were dissolved with 1.0 ml mobile phase of acetonitrile-water (30:70) by vortex motion, then transferred to a 1.5 ml Eppendorf tube for high-speed centrifugation at 13,000 x g for 20 minutes. Supernatant fluids of the solutions were collected for testing plasma concentrations of FFC by HPLC. According to the test data by HPLC, the peak area ratio (marked S) of FFC/CM was processed against the corresponding concentration (marked C) of FFC for regression analysis and determination of correlation coefficient (marked R). The FFC standard
solutions of 0.5 µg/ml, 10 µg/ml and 400 µg/ml were processed for recovery ratio, precision and the lowest limit of detection.

According to the micro-dilution method (Zhiying, 1985), the mycoplasma solution was diluted to 104 CCU/ml, while FFC was prepared at 100 µg/ml using acedimethylamide and propylene glycol. This process determined the MIC of FFC on separated strains of CCPP. The process was repeated three times for determination and the computation of the mean.

Data Analysis

The data of the present test was analyzed by 3P97 computer software; the best fit of pharmacokinetics model was judged by Weighted Sum of Squares (WSS) and Akaike Information Criterion (AIC) value; data of plasma concentration and pharmacokinetic parameters of FFC was analyzed with SPSS (12.0) software.

Results and discussion

Pathological Model Identification

Following fifteen days after being inoculated with the mycoplasma solution of CCPP, the goats among group B demonstrated the following clinical symptoms: fever, cough, serous nasal discharge, labored breathing, lethargy and dullness. The goats of Group B serologically tested positive for CCPP. The bacteria strain separated from the nasal discharge of the goats were cultivated to colonies in Hayflick’s solid medium and appeared to be similar to fried eggs with a bellybutton (Fig.1). Growth inhibition test demonstrated that the bacteria strain separated from the goats was sensitive to positive serum of CCPP. Biochemical test showed that it was sensitive to 1.5% foxglove saponin and could undergo fermentation utilizing glucose. Urease activity examination and arginine hydrolysis test were negative.

Regression test using separated bacteria strains showed similar clinical symptoms of CCPP, fifteen days after inoculation of mycoplasma solution of CCPP, and were positive by serological testing. Dissection and histological examination of the diseased goats showed pathological changes consistent with lung congestion and pleural adhesions, and thoracic cavity filled with small amounts of purulent liquor. Histological examination of lung tissue demonstrated pathological changes of suppurative bronchial pneumonia with accumulations of lymphocytes, neutrophils and plasma cells, and compensatory expansion of parts of the alveolus (Fig. 2).

According to these symptoms of pathological and colony features, biochemical identification test, growth inhibition test and animal regression analysis, the pathological model of CCPP in goats was identified.

Standard Curve and Chromatogram

The retention time tests of FFC and CM were 9.83 minutes and at 10.18 minutes, respectively; the chromatographic peak was sharp and symmetrical, with adequate separation from impurity peaks (Fig. 3 and 4).

The regression equation is:

\[
C = 3.645S - 0.356, \quad R^2 = 0.9957
\]

Where:

- \( C \) = Concentration of FFC in the blood;
- \( S \) = Peak area ratio of FFC/CM; and
- \( R^2 \) = Correlation coefficient.

The recovery ratio of FFC and CM were 100.82 ± 0.23% and 92.81 ± 0.03%, respectively. Intra-day precisions of the FFC standard solutions of 0.5 µg/ml, 10 µg/ml and 400 µg/ml were 4.1%, 2.0%, 1.6% respectively; and inter-day precisions were 5.1%, 0.7%, 0.9%, respectively. The lowest detection limit was 0.01 µg/ml. These numbers demonstrated that the method of the present study has excellent feasibility.
Pharmacokinetic Characteristics

The data of plasma concentrations and pharmacokinetic parameters of FFC in both groups of goats are shown in Table 1 to 4. Precision day were 5.1. Results of the study showed that the pharmacokinetic characteristics of intramuscular injection of FFC in healthy goats (Group A) was a one-compartment open model demonstrating complete absorption, quick distribution, high effective concentration and slow elimination. The fitting equation was:

\[ C = 6.57(e^{-0.11t} - e^{-2.56t}) \]

Where:
- \( C \) = Concentration of FFC in the blood; and
- \( t \) = time.

The main pharmacokinetic parameters are as follows:
- \( T_{1/2Ka} \) (half life of absorption) = 0.45 ± 0.29 h,
- \( T_{1/2ke} \) (half Life of Elimination) = 9.64 ± 4.78 h,
- \( T_{peak} \) (peak time of drug) = 1.80 ± 0.71 h,
- \( C_{max} \) (maximum concentration of drug) = 4.16 ± 1.23 mg/l,
- \( AUC \) = 60.34 ± 17.25 mg/h/l.

In the present study, when injecting the same dosage the absorption of FFC among healthy goats was similar to sheep (Xiubo et al., 2003), and slower than that of pigs (Jianzhong et al., 2003). The \( C_{max} \) value of 4.16...
mg/l in goats was close to that in sheep (4.2 mg/l) and higher than that of cows (3.07 mg/l) and pigs (3.2 mg/l) (Jianzhong et. al., 2003). The Tpeak of the goats (1.80 h) is earlier than that of cows (3.30 h), but later than that of sheep (1.38 h) and pigs (0.91 h).

Tab.2 Pharmacokinetic parameters of FFC after intramuscular injection in the control group (Group A)

<table>
<thead>
<tr>
<th>Parameter*</th>
<th>Unit</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>X ± s</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>mg L⁻¹</td>
<td>16.81 4.05 4.13 4.05 3.26 7.11</td>
<td>6.57±5.19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ke</td>
<td>h⁻¹</td>
<td>0.28 0.05 0.07 0.05 0.06 0.12</td>
<td>0.11±0.09</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ka</td>
<td>h⁻¹</td>
<td>0.80 2.33 7.25 2.72 1.14 1.15</td>
<td>2.56±2.41</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1/2α</td>
<td>h</td>
<td>0.01 0.01 0.00 0.02 0.01 0.01</td>
<td>0.01±0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1/2xe</td>
<td>h</td>
<td>2.49 14.38 9.51 14.02 11.87 5.59</td>
<td>9.64±4.78</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPEAK</td>
<td>h</td>
<td>2.03 1.70 0.64 1.50 2.74 2.17</td>
<td>1.70±0.71</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMAX</td>
<td>mg L⁻¹</td>
<td>6.21 3.65 3.90 3.69 2.64 4.84</td>
<td>4.16±1.23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC</td>
<td>mg h L⁻¹</td>
<td>39.21 82.20 56.05 80.47 53.51 51.11</td>
<td>60.3±17.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL/F(s) Lkgs⁻¹</td>
<td>0.51 0.24 0.36 0.25 0.38 0.39</td>
<td>0.35±0.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V/F(c) Lkg⁻¹</td>
<td>1.83 5.05 4.90 5.03 6.47 3.16</td>
<td>4.40±1.64</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tab.3 Plasma concentration of FFC after intramuscular injection in the treatment group (Group B)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Num. for the goats</th>
<th>X ± s</th>
</tr>
</thead>
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<tr>
<td>0.083</td>
<td>1.14 0.73 0.79 1.85 1.26 0.88</td>
<td>1.11±0.42</td>
</tr>
<tr>
<td>0.167</td>
<td>2.27 0.99 1.40 3.08 2.96 2.02</td>
<td>2.12±0.83</td>
</tr>
<tr>
<td>0.25</td>
<td>2.71 1.35 1.76 3.22 3.86 2.46</td>
<td>2.56±0.92</td>
</tr>
<tr>
<td>0.50</td>
<td>3.24 1.66 1.92 3.46 4.59 2.90</td>
<td>2.96±1.07</td>
</tr>
<tr>
<td>0.75</td>
<td>3.94 1.76 2.48 4.43 5.86 3.88</td>
<td>3.72±1.45</td>
</tr>
<tr>
<td>1</td>
<td>3.65 1.97 2.96 4.75 6.66 4.09</td>
<td>4.02±1.61</td>
</tr>
<tr>
<td>2</td>
<td>3.60 1.91 2.64 4.15 7.35 5.01</td>
<td>4.11±1.93</td>
</tr>
<tr>
<td>3</td>
<td>3.16 2.10 2.65 3.42 5.69 5.01</td>
<td>3.67±1.39</td>
</tr>
<tr>
<td>6</td>
<td>3.10 1.84 2.38 3.04 5.10 4.47</td>
<td>3.32±1.24</td>
</tr>
<tr>
<td>8</td>
<td>3.04 1.45 2.36 3.13 3.34 4.25</td>
<td>2.93±0.94</td>
</tr>
<tr>
<td>12</td>
<td>2.64 0.99 1.98 2.51 1.81 2.68</td>
<td>2.10±0.65</td>
</tr>
<tr>
<td>24</td>
<td>1.04 0.05 0.42 0.79 0.11 0.40</td>
<td>0.47±0.39</td>
</tr>
</tbody>
</table>

MIC and Effective Concentration

Results of the test showed that the value of MIC of FFC on the strain separated from mycoplasma of CCPP was 0.18 ± 0.07 µg/ml. It has been reported that the values of MIC of FFC on 243 strains of pathogenic bacteria separated from the respiratory tract of cows and pigs were from 0.12 to 4 µg/ml (Shin et al., 2005).

In the present study, the mean plasma concentration of FFC in CCPP goats was 0.82 µg/ml 24 hours following injection. This number is 4.6 times the value of MIC of FFC on mycoplasma strains of CCPP. The time of effective plasma concentration of FFC by intramuscular injection with a single dose of FFC at 20 mg/kg body weight will maintain more than 24 hours in goats.

Conclusions

There was no apparent difference in pharmacokinetic characteristics of FFC in healthy and CCPP goats. CCPP, as a respiratory tract disease, has no significant influence on pharmacokinetic characteristics of FFC. The parameters of clinical therapy scheme of FFC treatment in goats diagnosed with CCPP are: administration cycle (T), 24.21 h; first dose (X₀), 21.20 mg/kg; maintaining dose (X), 20 mg/kg; times of administration (n), 2.38 when the fractional number reaching plateau of 99.9%.

Literature Cited


Daihua, W., Gangyi Xu, 2005. Epidemic status and control technology of contagious caprine pleuropneumonia. Sichuan Animal Husbandry and Veterinary. 32(10), 48-49.


Smith, M.C., Sherman, D. M., 1994. Goat medicine, Lippincott Williams & Wilkins, Maryland, 256-257.

