Comparative Pharmacokinetics of Tylosin or Florfenicol after a Single Intramuscular Administration at Two Different Doses of Tylosin-Florfenicol Combination in Pigs

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(Received 11 June 2007/Accepted 7 September 2007)

ABSTRACT. Clinical pharmacokinetic profiles were investigated following intramuscular (i.m.) administration to pigs with a commercial tylosin-florfenicol combination product at a dose of 2.5 mg/kg tylosin and 5 mg/kg florfenicol or 10 mg/kg tylosin and 20 mg/kg florfenicol. The quantitation limit (QL) of florfenicol was 0.1 \( \mu g/mL \). The pharmacokinetic characteristics after i.m. doses were fitted by a one compartment open model. A fourfold decrease in the normal dose of each drug (20 mg/kg to 5 mg/kg for florfenicol, and 10 mg/kg to 2.5 mg/kg for tylosin) resulted in a corresponding two fold decrease in each drug of the maximum plasma concentration (\( C_{max} \)) and the area under curve (AUC) values.

KEY WORDS: florfenicol, pharmacokinetics, tylosin.

Tylosin is a macrolide antibiotic with bacteriostatic action against many gram-positive and anaerobic bacteria, mycoplasmas and some rickettsiae [10]. It interferes with protein synthesis by reversibly binding to the 50S subunit of the ribosome [17]. Because of its effectiveness against gram-positive bacteria, Mycoplasma spp. and Chlamydia spp., tylosin has been widely used for treatment of swine dysentery, arthritis, and pneumonia in pigs [9].

Pharmacokinetics of tylosin has been described in many species including cattle, buffaloes, sheep, goat, dogs and birds [8, 12, 15, 18]. Many bacteria of pig origin mainly, Mycoplasma hyopneumoniae, Bordetella bronchiseptica, Staphylococcus aureus and Erysipelothrix rhusiopathiae are reported to be sensitive to tylosin [14], and the response of pigs to tylosin are widely documented [9]. However, except one study [9], there has been no detailed investigation on the pharmacokinetics of tylosin in pigs.

Florfenicol, which belongs to the chloramphenicol family, is a novel broad-spectrum antibiotic for veterinary use. It has equal efficacy comparable to that of chloramphenicol, lower toxicity and less development of resistance [2]. In vivo and in vitro antibacterial activities of florfenicol against bovine and porcine respiratory pathogens have been described [7, 11]. Recently, florfenicol tends to be increasingly used for treating porcine pneumonias, the main target bacteria in pigs being \( P.\ multocida \), Actinobacillus pleuropneumoniae, Bordetella bronchiseptica and Streptococcus \( Suis \) [5, 11]. In the past years, florfenicol resistance has been detected in a wide variety of enteric bacteria, including various Salmonella enterica serovars, Klebsiella pneumoniae, Vibrio cholerae, and Escherichia coli [1]. There are also indications to the emergence of florfenicol-resistant \( P.\ multocida \) isolates [6].

Combination therapy with antimicrobial agents may be necessary to treat mixed bacterial infections, to achieve synergistic antimicrobial activity and to prevent the emergence of drug resistance. Generally, bacteriostatic agents act in additive fashion, whereas bactericidal agents are often synergistic. Although there is evidence that antagonism can occur in vitro with combinations of 50S subunit ribosomal inhibitors, there have been no clinical reports that these observations are relevant in vivo [3].

In this regard, the purpose of this study was to determine the pharmacokinetic profile of a commercial tylosin-florfenicol combination product (FTD-inj®, Shinilbiogen Co., Ltd, Korea) consisted of florfenicol 50 mg and tylosin tartrate 25 mg per ml following intramuscular (i.m.) administration to pigs, and also to compare the pharmacokinetic parameters of the drugs at 2 different doses.

In a 2 period study, a commercial tylosin-florfenicol combination product was used to inject 6 pigs at two different doses (tylosin 2.5 mg plus florfenicol 5 mg/kg body weight, and tylosin 5 mg plus florfenicol 20 mg/kg body weight). Drug concentration in the serum was determined by LC/MSD system, and the data obtained were subjected to one-compartment open model analysis by PCNONLIN (Version 4.2). The experiments were conducted on clinically healthy pigs (Landrace x yorkshire x Duroc), ranging from 35 kg to 42 kg of bodyweight.

For the analysis of the drugs in serum, florfenicol was extracted from the serum by van de Riet et al.’s method [16]. Tylosin was extracted from the serum by modification...
The mass spectrometer was tuned and optimized for the transmission of the nominal positive ion of florfenicol and tylosin, respectively. The optimal condition for the analysis of florfenicol and tylosin employed pneumatic nebulization with deionized water and 20% solution B (methanol) for florfenicol and 80% solution A (acetonitrile) for tylosin. The LOQ of florfenicol was 0.1 µg/mL, the inter-day and intra-day precision (CV %) was both below 10% and calibrations were linear (r>0.99) from 0.1 to 50 µg/mL. The LOQ of tylosin was 0.05 µg/mL, the inter-day and intra-day precision (CV %) was both below 10% and calibrations were linear (r>0.999) from 0.05 to 50 µg/mL.

Pharmacokinetic analysis of serum concentration-time data was made using a PCNONLIN compartmental model program (SCI Software, Clin Trials Company, Kentucky, U.S.A.). By visual inspection of drug concentrations plotted on semilogarithmic axes, a one-compartment open model was found to best describe the data. The paired t-test was used to test the effect of dose on parameter values after i.m. administration at two different doses.

The disposition of both drugs after i.m. doses could be described adequately by a one compartment model. The serum levels of both drugs at different doses could be detected early at 15 min postadministration. The pharmacokinetic parameters of tylosin and florfenicol following i.m. administration of the mixture at two different doses are presented in Table 1. The mean serum concentrations against time are illustrated in Fig. 1. The calculated maximum serum concentration (C_{max}) values (5.93 ± 1.20 and 2.71 ± 1.09 µg/mL) were achieved at 2.27 ± 0.55 and 2.57 ± 1.03 hr for florfenicol and tylosin at the normal recommended doses of 20 and 10 mg/kg respectively.

In our study, a fourfold decrease in the normal dose of each drug (20 mg/kg to 5 mg/kg for florfenicol, and 10 mg/kg to 2.5 mg/kg for tylosin) resulted in a corresponding two fold decrease in each drug of the maximum serum concentration (C_{max}) and the AUC values. The time to reach the maximum serum concentration (T_{max}), absorption and terminal half lives (T_{1/2abs} and T_{1/2el}) were, however, unaffected by change in the dose of florfenicol. T_{max} was not affected by change in the dose of tylosin, whereas a slightly faster absorption at lower dose (1.14 hr) than the normal dose (1.36 hr) was observed.

Statistical comparison of the parameters between the two dose levels revealed significant difference in the AUC and C_{max} of florfenicol (P<0.01, t-test), whereas all other parameters were almost similar in the two dose levels, notwithstanding the fact that the values were different for individual animals between the two doses.

Attempts were made to compare the present findings with data obtained previously after IM administration of florfenicol alone to pigs (data not presented). The major pharmacokinetic values were significantly lower in the combination therapy than administration of florfenicol alone. No significant difference in all pharmacokinetic parameters was observed between the two dose levels of tylosin. The experimental data in this study was best fitted to a one compartment model in accordance with previous reports in different animals [7, 13, 15], though other authors preferred a non compartmental model for the same route of administration [5, 9]. The elimination half life of tylosin 10 mg/kg in this study (3.01 ± 2.77 hr) is higher than the reported figures in cattle and buffaloes (2.24 and 2.4 hr, respectively) [12], but significantly lower than observations in pig [9], in the latter the mean elimination half life (T_{1/2el}) of five pigs was calculated to be greater than 24 hr. The differences may account for different commercial preparations, although the effect of

### Table 1. Pharmacokinetic parameters of tylosin-florfenicol combination after intramuscular administration at two different doses in pigs

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Tylosin</th>
<th>Florfenicol</th>
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<tbody>
<tr>
<td></td>
<td>2.5 mg/kg</td>
<td>10 mg/kg</td>
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<tr>
<td>AUC_{0-∞} (µg·h/mL)</td>
<td>14.1 ± 17.8</td>
<td>25.8 ± 27.1</td>
</tr>
<tr>
<td>C_{max} (µg/mL)</td>
<td>1.31 ± 0.49</td>
<td>2.71 ± 1.09</td>
</tr>
<tr>
<td>T_{max} (hr)</td>
<td>2.36 ± 0.99</td>
<td>2.57 ± 1.03</td>
</tr>
<tr>
<td>K_{01} (hr)</td>
<td>0.74 ± 0.31</td>
<td>0.59 ± 0.27</td>
</tr>
<tr>
<td>K_{10} (hr)</td>
<td>0.33 ± 0.15</td>
<td>0.32 ± 0.16</td>
</tr>
<tr>
<td>T_{1/2abs} (hr)</td>
<td>1.14 ± 0.62</td>
<td>1.36 ± 0.49</td>
</tr>
<tr>
<td>T_{1/2el} (hr)</td>
<td>3.88 ± 4.87</td>
<td>3.01 ± 2.77</td>
</tr>
</tbody>
</table>

Data values are expressed as Mean ± SD (n=6), * P<0.01. AUC_{(0-∞)} area under the serum concentration time curve from time zero to infinity; C_{max} and T_{max} peak serum concentration and time to required to attain peak concentration, respectively; K_{01} and K_{10} absorption and elimination rate constants, respectively; T_{1/2abs}: absorption half-life; T_{1/2el}: elimination half-life.
one drug on the kinetic properties of the other is also a subject of further study. Consistent with a similar study in broiler chicken [13], change in the dose of florfenicol had no effect on $T_{\text{max}}$. However, the changes in the peak concentration of both drugs were not proportional to changes in dose, in which a relatively lower absorption of both drugs was observed at higher doses. A similar non-proportional increase of $C_{\text{max}}$ with change in dose of oral florfenicol was observed in broiler chicken [13]. The lower peak concentration and AUC seen at higher concentration may concern residues at the injection site, though we are at the early stage to support with data the idea that residues should be considered more in combination therapy than in single therapy. The calculated value of AUC for the 20 mg/kg dose of florfenicol (60.9 hr $\mu$g/ml) is almost comparable to previous report in pigs (68.61 hr $\mu$g/ml) [7], but is lower than another study (84.3 hr $\mu$g/ml) [5]. Other parameters as $C_{\text{max}}$, $T_{\text{max}}$, $T_{1/2\text{ abs}}$, and $T_{1/2\text{ eli}}$ are significantly lower than previous reports by Liu et al. (2003) and Jiang et al. (2006) [5, 7]. The differences in these parameters may be due to the use of different commercial preparations.

In summary, the pharmacokinetic parameters of many antibiotics have been calculated after administration with a single antibiotic. However, a lot of antibiotics have been formulated as mixtures of more than two antibiotics in clinical area. Therefore, it might be valuable to assume here that the pharmacokinetic parameters and serum concentration of tylosin and florfenicol may give baseline information in the ratio of combination to be formulated for a product. These findings would be helpful in designing a rational dosage regimen. In advance, we need a further work on other kinetic aspects, tissue residues, and killing rate curve in vitro, according to the various ratios of two antibacterial combinations for determination of formulation in pigs.

ACKNOWLEDGMENTS. This work was supported in part by Shinilbiogen Co., Ltd. Brain Korea 21 Project and in part by Ministry of Commerce, Industry and Energy (MOCIE) through the Center for Traditional Microorganism Resources (TMR) at Keimyung University. Elias Gebru and Zhi-Qiang Chang was supported by a grant from the Brain Korea 21 Project.

REFERENCES