Comparative bioavailability between two routes of administration of florfenicol and flunixin in cattle

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SUMMARY

Resflor® is a fixed combination of two active ingredients: florfenicol and flunixin. The purpose of this study was to document the bioequivalence of two routes of administration namely the test intramuscular (IM) and the reference subcutaneous (SC) route of administration in cattle. Ten healthy female cattle were randomly allocated to one of the two groups of a two-period crossover design with a washout interval of 15 or 16 days between the two periods. The tested doses for the two routes of administration were 40 mg/kg BW for florfenicol and 2.2 mg/kg BW for flunixin. Blood samples were regularly collected up to 5 days following administration. Florfenicol and flunixin were assayed using HPLC analytical techniques. The limits of quantification (LOQ) were 0.05 µg/mL and 0.012 µg/mL for florfenicol and flunixin respectively allowing quantifying florfenicol plasma concentrations over 5 days and flunixin plasma concentrations over 24-58h post administration. Based on the regulatory criterion requiring that the 90% confidence interval of the ratio of the two pivotal pharmacokinetic parameters for florfenicol (AUC and Cmax) needs to be totally included in an a priori equivalence interval of ± 20%, it was concluded that the two routes of administration cannot be considered as bioequivalent. Indeed, the mean differences were as high as 35% and 63% for the AUC and Cmax respectively, the IM route administration leading to higher florfenicol exposure than the reference SC route of administration. In contrast, the kinetic disposition of flunixin was rather similar for the two routes of administration. 

Keywords: Bioequivalence, routes of administration, florfenicol, flunixin, cattle.

RÉSUMÉ

Etude comparative de biodisponibilité entre deux voies d’administration, du florfenicol et de la flunixine chez des bovins

Le Resflor® est la combinaison de deux principes actifs : le florfenicol et la flunixin. Le but de cette étude était de documenter la bioéquivalence de deux voies d’administration ; la voie intramusculaire (test) et la voie sous-cutanée (référence) chez les bovins. Dix génisses ont été aléatoire réparties dans un des deux groupes selon une étude croisée à deux périodes avec un intervalle de 15 ou 16 jours entre les deux périodes. La dose évaluée pour les deux voies d’administration était de 40 mg/kg pour le florfenicol et de 2,2 mg/kg pour la flunixin. Les échantillons de sang ont été régulièrement collectés pendant 5 jours après l’administration. Le florfenicol et la flunixin ont été dosés par HPLC. La limite de quantification (LOQ) était 0,05 µg/mL et 0,012 µg/mL pour le florfenicol et la flunixin permettant respectivement de quantifier des concentrations plasmatiques de florfenicol pendant plus de 5 jours et des concentrations plasmatiques de flunixin pendant 24-58 h après administration. En considérant les critères réglementaires sur les études de bioéquivalence, les deux voies d’administration ne peuvent pas être considérées comme bioéquivalentes car la différence entre les moyennes de l’AUC et du Cmax sont respectivement supérieures à 35 % et 63 %. L’administration par voie IM conduisant à une exposition plus élevée pour le florfenicol que l’exposition par voie SC (référence). En revanche, l’exposition plasmatique de la flunixin est similaire pour les deux voies d’administration.

Mots clés : Bioéquivalence, voie d’administration, florfenicol, flunixin, bovin.

Introduction

Intramuscular (IM) and subcutaneous (SC) routes of drug administration are routinely used in veterinary medicine and they are often considered as more or less equivalent. Actually these two routes of administration do not necessarily lead to the same plasma concentration profiles and this may be acknowledged for some classes of drugs such as antibiotics for which efficacy of a given formulation or route of administration is related not only to the corresponding overall bioavailability, but also to the shape of the plasma concentrations vs. time curve. The IM route generally leads to higher area under the plasma vs. time curve (AUC) and maximal plasma concentration (Cmax) values than the SC route and IM route may be preferred to optimise a concentration dependent antibacterial action. Conversely, a SC route of administration generally leads to a longer time above the minimal inhibitory concentration (MIC) of the pathogen and the SC route may be preferred to optimise a time dependent antibacterial action.

Florfenicol is a broad-spectrum time-dependent antimicrobial agent. Florfenicol is indicated in the treatment of bacterial pneumonia and associated respiratory infections in cattle and swine [1, 2]. The recommended dosage regimen for bovine is either a single dose of 40 mg/kg BW by SC route or two doses of 20 mg/kg BW by IM route with an interval of 48h apart. Recently a new formulation containing florfenicol associated with flunixin meglumine was developed for use in the treatment of infections in cattle and swine. Flunixin is a nonsteroidal anti-inflammatory drug (NSAID) frequently associated with antimicrobial agents to prevent irreversible lung lesions associated with pneumonia. This fixed association is marketed (Resflor®, Intervet/Schering-Plough) as a single subcutaneous injection for cattle but information on the IM route of administration are lacking.
The purpose of the present study was to compare the plasma kinetic profile of florfenicol and flunixin after either a single IM or a single SC injection in cattle of a commercial formulation containing both flunixin and florfenicol, in order to support either a bioequivalence of the two routes of administration or rather the possible selective use of IM or SC route of administration for this new formulation.

Materials and Methods

EXPERIMENTAL DESIGN AND ANIMALS

Ten healthy non pregnant cycling female cattles having a body weight between 437 and 563 kg and aged approximately 2.8 years old were randomly administered by the IM and SC route of administration according to a two-period cross-over design with 15 or 16-days washout time between periods.

The necessary volume of the commercial Resflor® formulation (Intervet/Shering Plough Animal Health, Friesoythe Germany) was calculated considering body weights of cattle. The dose was 40 mg/kg BW for florfenicol and 2.2 mg/kg BW for flunixin (administered as meglumine) i.e. 2 mL per 15 kg BW of the commercial solution. The dose was administered in the neck muscle for the IM administration and under the loose skin of the left shoulder for the SC administration. As the maximal volume recommended per injection site was <10 mL, the total dose was split into 6-8 injection sites on the left of the neck.

BLOOD SAMPLING

Blood samples were regularly collected from the jugular vein up until 120 hours post administration. The samples were immediately chilled in ice and centrifuged at about 3000 g within 2 hours following sampling. The plasma was stored at -20°C.

ANALYTICAL METHOD AND VALIDATION PROCEDURE

Plasma florfenicol and flunixin concentrations were determined separately by using a validated high performance liquid chromatography (HPLC) method with ultra violet (UV) detection.

Florfenicol analysis

Plasma samples were extracted by solid phase extraction (SPE) on C8 cartridges. Fifty µL of the residue were injected on an analytical C18 column (ODS-3 150x4.0mm; 3µm, Interchim, Montluçon – France). The mobile phase was a 76/24 mixture H2O/AcN with a flow rate of 0.8 mL/min and a detection at \( \lambda_{\text{abs}} = 225 \) nm.

Flunixin analysis

Plasma samples were extracted by SPE (HLB cartridges) and 50 µL of the extract were injected onto the HPLC system at 0.6 mL.min\(^{-1}\) flow rate, on a 30/70 AcOH(1%)/MeOH mixture and a detection at \( \lambda_{\text{abs}} = 284 \) nm.

Validation results. Each analytical method was validated according to the FDA guidelines [3]. Results were summarized in the Table 1.

All unknown samples from a single animal (IM and SC kinetics) were simultaneously processed with the same daily calibration curve. The daily calibration curve was validated by back calculation and when at least 4 of the 6 calculated QC were lower than 20% of their nominal values.

PHARMACOKINETICS AND STATISTICAL ANALYSIS

A non-compartmental analysis (NCA) of florfenicol and flunixin plasma concentration profiles was performed using model 200 of WinNonlin (WinNonlin Professional, version 5.2, Pharsight Corporation, Mountain View, CA). The area under the plasma concentration-time curve was calculated by the linear trapezoidal rule from 0 to the last measured plasma concentration.

Statistical calculations were done using the appropriate descriptive statistical WinNonlin tool associated with the NCA. Comparison of pharmacokinetic parameters (IM vs. SC) was carried out using paired "t" test (SYSTAT version 10, SPSS Inc.).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Florfenicol</th>
<th>Flunixin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration range (µg/mL)</td>
<td>0.05-10</td>
<td>0.012-1.2</td>
</tr>
<tr>
<td>Internal standard</td>
<td>Chloramphenicol</td>
<td>Ketoprofen</td>
</tr>
<tr>
<td>Model of the calibration curve</td>
<td>Linear, weighting 1/X²</td>
<td>Quadratic, weighting 1/X</td>
</tr>
<tr>
<td>Mean recovery</td>
<td>98 %</td>
<td>55 %</td>
</tr>
<tr>
<td>Precision-repeatability (CV%)</td>
<td>7%</td>
<td>11%</td>
</tr>
<tr>
<td>Precision-reproducibility (CV%)</td>
<td>11%</td>
<td>14%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>93-100%</td>
<td>95-102%</td>
</tr>
<tr>
<td>Limit of quantification (µg/mL)</td>
<td>0.05</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Table I: Results of the validation method for florfenicol and flunixin.
Results and Discussion

FLORFENICOL

Mean (+SD) arithmetic and semilogarithmic plots of florfenicol plasma concentration (µg/mL) vs. time (h) after an IM and a SC administration of the formulation for the 10 cattle are shown in figure 1 and 2. Visual inspection of the curves clearly indicates that plasma florfenicol concentrations reached higher values as expected after an IM administration than after a SC administration of the formulation (figure 1Legends) and it is shown that the slopes of the terminal phase are different for the IM and SC routes of administration (figure 2). After the SC administration of the formulation, the plasma florfenicol concentrations decreased more slowly than after the IM administration explaining that after a period of time of about 48h, the mean plasma florfenicol concentrations were systematically higher after the SC than after the IM florfenicol administration.

Mean pharmacokinetic florfenicol parameters as obtained from the NCA are given in table 2. The relative bioavailability of florfenicol was increased by approximately 35% for the IM over the SC route of administration. Cmax was higher after the IM administration with a relative increase of about 63%. These results indicate that the IM and SC routes cannot be bioequivalent because the a priori equivalence interval is of ± 20%. The value of the terminal half-life that was significantly longer after the SC than the IM administration (39.6±15.6h vs. 25.5±4.6h for the SC and IM route of administration respectively, \( P=0.032 \)). After an IV florfenicol administration either in lactating cow or in calf, the reported terminal half-life of florfenicol was about 3h [4-6] i.e. much shorter than the terminal half-life observed in the present trial. This indicates that for both IM and SC route of administration for Resflor®, the terminal phase corresponds to a slow process of florfenicol absorption (flip-flop kinetics) [7].

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>Route of administration</th>
<th>Florfenicol mean±SD</th>
<th>Flunixin mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUClast</td>
<td>µg*h/mL</td>
<td>IM</td>
<td>224.66 ± 44.77</td>
<td>10.25 ± 4.05</td>
</tr>
<tr>
<td>AUClast</td>
<td>µg*h/mL</td>
<td>SC</td>
<td>164.2± 32.02</td>
<td>9.80± 2.99</td>
</tr>
<tr>
<td>Cmax</td>
<td>µg/mL</td>
<td>IM</td>
<td>8.09 ± 1.98</td>
<td>2.70 ± 1.21</td>
</tr>
<tr>
<td>Cmax</td>
<td>µg/mL</td>
<td>SC</td>
<td>4.96± 1.76</td>
<td>1.37± 1.03</td>
</tr>
<tr>
<td>Tmax</td>
<td>h</td>
<td>IM</td>
<td>5.35 ± 2.91</td>
<td>0.33 ± 0.12</td>
</tr>
<tr>
<td>Tmax</td>
<td>h</td>
<td>SC</td>
<td>7.63± 2.07</td>
<td>1.60± 2.52</td>
</tr>
<tr>
<td>T1/2</td>
<td>h</td>
<td>IM</td>
<td>25.4± 4.57</td>
<td>5.18 ± 0.98</td>
</tr>
<tr>
<td>T1/2</td>
<td>h</td>
<td>SC</td>
<td>39.6± 15.62</td>
<td>7.46± 2.61</td>
</tr>
<tr>
<td>MRTlast</td>
<td>h</td>
<td>IM</td>
<td>27.5± 2.94</td>
<td>5.96 ± 1.09</td>
</tr>
<tr>
<td>MRTlast</td>
<td>h</td>
<td>SC</td>
<td>36.14± 4.63</td>
<td>9.36± 1.80</td>
</tr>
</tbody>
</table>

\( AUC_{\text{last}} \): area under the plasma concentration vs. time curve (µg*h/mL) calculated by trapezoidal rule from 0 to the last observed concentration

\( C_{\text{max}} \): peak plasma concentration (µg/mL)

\( T_{\text{max}} \): time (h) to reach \( C_{\text{max}} \) after drug administration

\( T_{1/2} \): terminal half-life (h)

\( MRT_{\text{last}} \): mean residence time computed from 0 to the last observed concentration

Mean ± SD: arithmetic mean ± standard deviation

Statistical significance between IM and SC route of administration (paired "t" test).

\( 'P<0.001; 'P = 0.002; 'P>0.05; 'P = 0.032; 'P<0.001 \)

Table II: Pharmacokinetic parameters of florfenicol and flunixin after an intramuscular (IM) or a subcutaneous (SC) administration of the florfenicol (40 mg/kg) and flunixin (2.2 mg/kg) dose in 10 cattle.
Such a flip-flop was already reported by others with a terminal half-life of florfenicol of 18h after an IM florfenicol administration [8]. Such a slow process of absorption is a definitive advantage for a time-dependent antibiotic allowing maintaining relevant plasma florfenicol concentrations over a long period of time after a single dose administration. At 48h post florfenicol administration, the plasma florfenicol concentrations were similar for the two investigated routes of administration (1.2±0.29 vs. 1.05±0.14 µg/mL for the IM vs. SC route of administration respectively). Beyond this delay, the plasma florfenicol was higher after the SC than after the IM administration. At 72h (3 days) post administration, the florfenicol plasma concentration (0.58±0.16 vs. 0.76±0.20 µg/mL for the IM and the SC route of administration respectively) were still therapeutically relevant against some pathogens (e.g. the MIC90 for Pasteurella multocida is 0.5µg/ml) [9].

**FLUNIXIN**

Figure 3 shows the semi-logarithmic plot of the mean (+SD) plasma flunixin concentration (µg/mL) vs. time (h) after an IM and a SC administration of the flunixin dose: 2.2 mg/kg as flunixin meglumin in ten cows (mean + SD). A similar value was reported in cow (8.12h) [11]. The reported terminal half-life of flunixin was 6.47±0.49h with no therapeutic meaning. After an IV flunixin administration in calf, the plasma concentration (0.58±0.16 vs. 0.76±0.20 µg/mL for the IM and the SC route of administration respectively) were still therapeutically relevant against some pathogens (e.g. the MIC90 for Pasteurella multocida is 0.5µg/ml) [9].

Values of the terminal half-lives were statistically different for the two routes of administration (5.18±0.98 vs. 7.46±2.61h for the IM and SC route of administration respectively, \(P=0.02\)) but the differences are without therapeutic meaning. After an IV flunixin administration in calf, the reported terminal half-life of flunixin was 6.47±0.49h [10]; a similar value was reported in cow (8.12h) [11]. The values obtained in the present experiment were very similar to those following an IV flunixin administration in cattle suggesting that flunixin, contrary to florfenicol, is rapidly absorbed either after an IM or a SC administration.

**Conclusion**

The plasma kinetic profiles of florfenicol after the two investigated routes administration IM and SC were not equivalent in these conditions. The IM route of administration leads to higher plasma florfenicol exposure than the SC route of administration and IM route of administration may be preferred for less susceptible pathogens; conversely, for a more susceptible pathogen, the SC route may be preferred as it maintains higher plasma florfenicol concentrations than the IM route 48h after injection.

**Acknowledgement**

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**References**