Short communication

Pharmacokinetics of levosulpiride after single-dose administration by different routes in sheep (Ovis aries Linnaeus)

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ABSTRACT

Levosulpiride (LSP) is the (−)-enantiomer of sulpiride and might represent a valid alternative to the current drugs used for the synchronization in small ruminants. The aim of this study was to provide the pharmacokinetic profile of LSP after intravenous (IV), intramuscular (IM) and oral (PO) administration in sheep. Six healthy female sheep underwent a randomized crossover study design with a washout period of 1 week. Each animal at the completion of the study received 50 mg of LSP by IV, IM and PO administrations. Plasma samples were collected prior and up to 24 h and, after the extraction procedure, samples were analysed by HPLC with spectrofluorometric detection. LSP concentrations were quantifiable until 10 and 8 h after IV and IM administration, respectively. After PO administration plasma concentrations were low and quantified until 4 h in all the animals. Clearance (121.5 ml/Kg) was fast and volume of distribution (241 ml/Kg h) small; half-life was short and very similar after both IV (1.80 h) and IM (1.66 h) administrations. The bioavailability after IM and PO was high (about 70%) and extremely low (about 6%), respectively. IV and IM groups showed a good correlation between AUC and the LSP dose expressed in mg/Kg, but very low correlation was found for the PO route. In conclusion, PO administration of LSP is not recommended in sheep while IV and IM administration show comparable PK profiles.

1. Introduction

Levosulpiride (LSP) is the (−)-enantiomer of sulpiride, an anti-psychotic drug used in human medicine primarily in the management of the symptoms of schizophrenia, senescence, depression, and other psychiatric disorders (Mucci et al., 1995). As its parent compound sulpiride (racemate), LSP antagonizes pre- and post-synaptic D2 and D3 receptors at striatum or nucleus accumbens (Rossi and Forgione, 1995; Mucci et al., 1995). LSP has shown however a lower acute toxicity when compared to sulpiride and (+)-enantiomer (Rossi and Forgione, 1995; Mucci et al., 1995). In veterinary medicine pharmacological treatments have been proposed for the synchronization of ovulation phase. Administrations of dopamine antagonists such as sulpiride in mares resulted in a hastening of first oviulations without interference on fertility (Panzani et al., 2011). Other studies described sulpiride pharmacokinetics on mares, horses and rabbits (Fiorica et al., 2015; Giorgi et al., 2013, 2015). In small ruminants LSP might represent a valid alternative to the current drugs used for the synchronization such as progesterone, prostaglandins and analogues, and melatonin (Hansel and Convey, 1993; McCracken et al., 1972; Rubianes et al., 2003; Abecia et al., 2012; Walkden-Brown et al., 1999). LSP pharmacokinetics have been recently tested in goats (Łebkowska-Wieruszewska et al., 2019), but at the best of authors’ knowledge no pharmacokinetic data have been reported in sheep. Hence, the aim of this study was to assess the pharmacokinetics of LSP after intravenous (IV), intramuscular (IM) and oral (PO) administration in sheep at a dosage of 50 mg.

2. Materials and methods

2.1. Animal treatment and sampling

Six healthy female sheep (Swiniarka breed) with BW ranging from 27.2 to 39.0 Kg (age 5–8 years) were used in the present study. These animals were selected in a flock of 600 animals in order to obtain sheep
were lower than 20% of AUC₀−∞ and R² (square of coefficient of determination) versus concentration. The dose was administered with 50mg of LSP (Levopraid, 50mg/2ml injectable solution). A wash out period was observed among the phases. Group A was administered with 50 mg of LSP (Levopraid, 50 mg/2 ml injectable solution, Teopharma) via injection into the right jugular vein. Group B received the same dosage by IM injection in the middle quadrant of the gluteus muscle. Group C orally received one 50 mg tablet of Levopraid (Teopharma), followed by an oral flush with 20 ml tap water to ensure complete delivery of the drug into the stomach. For all the groups feed was withheld 8 h before LPS treatment. Once the cross over study was completed each animal has been administered by each of the three routes. Blood samples were withdrawn from a pre-implanted catheter into the right jugular vein. A 5 ml aliquot of blood was collected by vacuum container containing lithium heparin at 0, 15, 30, 45 min and 1, 1.5, 2, 4, 6, 8, 10, and 24 h after each administration. The blood samples were immediately placed in ice, centrifuged at 1500x g and the harvested plasma stored at −20 °C until analysis.

2.2. Instrumentation and chromatographic conditions

The HPLC-FL system was an LC system (Jasco, Japan). Chromatographic separation assay and plasma extraction procedure was based on a previously reported method (Lebkowska-Wieruszewska et al., 2019). The methods were shortly revalidated according to the EMA guidelines (Anonymous, 2009) using sheep plasma. The calibration curve of peak area versus concentration (ng/ml) of LSP was plotted using data (in triplicate) from 7 concentration points (range 50–5000 ng/ml). Limit of detection (LOD) and limit of quantification (LOQ) were determined as analyte concentrations giving signal-to-noise ratios of 3 and 10, respectively.

2.3. Pharmacokinetics and statistical analysis

The pharmacokinetic calculations were carried out using ThothPro™ 4.2 software, (ThothPro™, www.thothpro.com). LSP plasma concentration versus time curves were modelled for each subject using a non-compartmental approach.

Maximum concentration (Cmax) of LSP and time required to reach Cmax (Tmax) were read from the data. The elimination half-life (T1/2) was calculated using nonlinear least squares regression analysis of the concentration-time curve, and the area under the concentration vs time curve (AUC₀−∞) was calculated with the logarithmic trapezoidal method and with the linear-up log-down rule for the IV and EV (IM and PO) administrations, respectively. From these values, the apparent volume of distribution at steady state (Vis = dose × AUMC/AUC²), mean residence time (MRT = AUMC/AUC) and systemic clearance (CL = dose/AUC) were determined. Pharmacokinetic estimates were calculated only if the individual values between AUC₀−∞ and AUC₀-t were lower than 20% of AUC₀−∞, and R² (square of coefficient of determination) of the terminal phase regression line was > 0.85.

The IM and PO F% were calculated using the following formula:

\[
F% = \frac{(AUMC_{IM} or PO) / AUC_{IV})}{100}
\]

The extraction ratio (E) for LSP in sheep after IV administration was calculated according to the formula:

\[
E = \frac{CL}{Q'}
\]

where CL is the value of clearance reported for each animal after IV administration, while Q’ (ml/min) is the cardiac output calculated according to the allometric equation:

\[
Q' = 180 \times \frac{BW^{-0.19}}{}
\]

where BW stands for body weight (Kg) of each animal (Toutain and Bousquet-Melou, 2004).

Pharmacokinetic variables were evaluated using the student’s t-test to determine statistically significant differences between groups. Pharmacokinetic parameters are presented as means ± SD (normality tested by Shapiro-Wilk test) and median and range. Differences were considered significant if p < 0.05. All analyses were conducted using GraphPad Prism (GraphPad Software, La Jolla, CA, USA).

3. Results

The method validation parameters resulted well within the limits requested from the guidelines for the analytical method validation (Anonymous, 2009) (Table 1).

A licensed veterinarian (B L-W) evaluated the animals’ health. They did not exhibit immediate or delayed (up to 7 days) visible local or systemic adverse effects. Fig. 1 displays the mean LSP plasma concentration vs time curves. Table 2 reports the main pharmacokinetic estimates for all the routes of administration. Extraction ratio was low (2.18 ± 0.11%). After PO administration, due to the small number of time points in which the LSP concentration was quantified, the calculation of most of the estimates was not possible. Fig. 2 displays the correlation between AUC and the LSP dose expressed in mg/Kg administered in each single animal in order to assess the coefficient of determination, estimated intercept and slope.

4. Discussion

The LSP dose range used in the present study (1.28–1.84 mg/Kg; mean 1.51 mg/Kg) was selected within the human clinical dose range (50–100 mg/day) (Gong et al., 2014). The doses used in sheep were also in line with the range (0.5–2 mg/Kg) reported in early studies on its use.
Duchamp and Daels, 2002; Giorgi et al., 2013, 2015; Guillaume et al., 2019). The racemate sulpiride to stimulated ovulation/lactation and to evaluate the pharmacokinetics in veterinary species (Daels et al., 2000; Wiesel et al., 1980). T1/2λz was lower than those reported for donkeys, (Giorgi et al., 2013, 2015). The differences in this parameter with equine species can be due to different cardiac output among the species (Toutain and Bousquet-Melou, 2004). How- ever, further PK/PD studies are needed to understand the clinical effective dose of LSP in sheep and if this estimate effective concentration can be reliable.

**Declaration of Competing Interest**

None.

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None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of this paper. This

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**Table 2**

Main LSP pharmacokinetic estimates after IV, IM and PO administration of 50 mg in sheep.

| Parameter | Unit | IV | Mean | SD | Median | Range | IM | Mean | SD | Median | Range | PO | Mean | SD | Median | Range
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</thead>
<tbody>
<tr>
<td>λz</td>
<td>1/h</td>
<td>0.39</td>
<td>0.036</td>
<td>0.38</td>
<td>0.34–0.45</td>
<td></td>
<td>0.42</td>
<td>0.05</td>
<td>0.41</td>
<td>0.37–0.50</td>
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<tr>
<td>T1/2 λz</td>
<td>h</td>
<td>1.8</td>
<td>0.16</td>
<td>1.82</td>
<td>1.54–2.02</td>
<td></td>
<td>1.66</td>
<td>0.19</td>
<td>1.69</td>
<td>1.40–1.88</td>
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<tr>
<td>Cmax</td>
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<td>4332.83</td>
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<td>Tmax</td>
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<td>0.25</td>
<td>0</td>
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<tr>
<td>AUC (0–last)</td>
<td>ng/ml</td>
<td>12518</td>
<td>1037</td>
<td>12466</td>
<td>11096–14033</td>
<td></td>
<td>8889</td>
<td>1086</td>
<td>9041</td>
<td>7519–10370</td>
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<tr>
<td>AUC (0–inf)</td>
<td>ng/ml</td>
<td>12699</td>
<td>1091</td>
<td>12641</td>
<td>11206–14305</td>
<td></td>
<td>9000</td>
<td>1077</td>
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<td>7627–10462</td>
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<td>CL</td>
<td>ml/Kg</td>
<td>121.5</td>
<td>9.89</td>
<td>120.5</td>
<td>110–137</td>
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<td>241</td>
<td>26</td>
<td>238.5</td>
<td>231–282</td>
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<tr>
<td>Vs</td>
<td>ml/Kg h</td>
<td>241</td>
<td>26</td>
<td>238.5</td>
<td>231–282</td>
<td></td>
<td>70.73</td>
<td>3.49</td>
<td>72.19</td>
<td>70.50–71.83</td>
<td>5.94</td>
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<tr>
<td>MRT</td>
<td>h</td>
<td>2.13</td>
<td>0.09</td>
<td>2.13</td>
<td>2.02–2.27</td>
<td></td>
<td>2.185</td>
<td>0.054</td>
<td>2.19</td>
<td>2.11–2.27</td>
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<td>F</td>
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<td>70.73</td>
<td>3.49</td>
<td>72.19</td>
<td>70.50–71.83</td>
<td>5.94</td>
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Abbreviations: λz, terminal phase constant; T1/2 λz, terminal half-life; Cmax, peak plasma concentration; Tmax, time of peak concentration; AUC(0–last), area under the plasma concentration-time curve from 0 to last time collected samples; AUC(0–inf), area under the plasma concentration-time curve from 0 to infinity; CL, clearance; Vs, volume of distribution at the steady state; MAT, mean absorption time; E, Extraction ratio; MRT, mean resident time; F, bioavailability. a,b,c Significantly different (p < 0.05) from IV, IM and PO group, respectively.

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**Fig. 2.** Coefficient of determination (R²) and estimated intercept and slope between weight-adjusted LSP doses and AUC (IV – ●; IM – ○; PO – □).
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References


