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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 cross over study design with a wash out 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 express 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 antipsychotic drug used in human medicine primary 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 ovulations 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 floc of 600 animals in order to obtain sheep

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with a body weight closest to those reported for goats in Łebkowska-Wieruszewska et al. (2019) study. The animals were determined to be clinically healthy based on a physical examination and full chemistry and haematological analyses. Animal experiments were conducted at the University of Life Science, Lublin, Poland. Animal care and handling was performed according to the provision of the Directive 2010/63/EU on the protection of animals used for scientific purposes. The animals were randomly divided in to three groups (A, $n = 2$; B, $n = 2$; C, $n = 2$) and underwent a cross over study design (3 × 3 Latin-square). A 1 week 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 vacutainer 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 30 min 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 (Łebkowska-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 ($T_{1/2}\lambda z$) was calculated using nonlinear least squares regression analysis of the concentration-time curve, and the area under the concentration *vs* time curve ($AUC_{0\text{-}inf}$) 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 (Vss = dose × AUMC/AUC^2), mean residence time (MRT = AUMC/AUC) and systemic clearance (CL = dose/AUC) were determined. Pharmacokinetic estimates were calculated only if the individual values between $AUC_{0\text{-}\infty}$ and $AUC_{0\text{-}t}$ were lower than 20% of $AUC_{0\text{-}\infty}$ and R^2 (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:

$$F\% = (AUC_{IM\ or\ PO})/(AUC_{IV}) \times 100$$

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

$$E = CL/Q^{\circ}$$

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:

$$Q^{\circ} = 180\ BW^{-0.19}$$

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).

Table 1
Main validation parameters of the analytical HPLC method used for the quantification of LSP in sheep plasma.

Parameter	Unit	
Inter-day	%	4.5–6.9
Intra-day	%	3.1–7.8
LOQ	ng/ml	50
LOD	ng/ml	20
Recovery	%	72–94

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 slop.

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

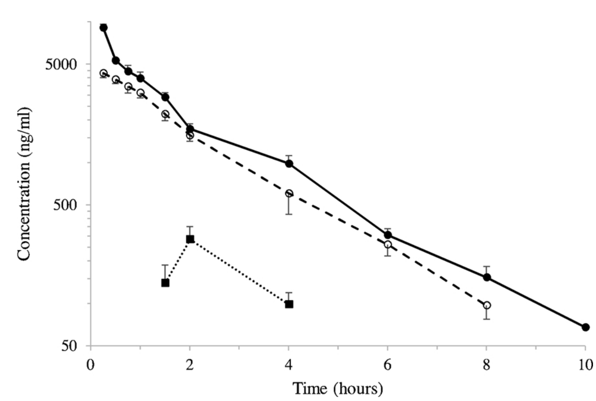

Fig. 1. Observed mean plasma concentration of LSP after IV (-●-), IM (–o–) and PO (··■··) administration of 50 mg in sheep. Vertical bars represent the standard deviation.

Table 2
Main LSP pharmacokinetic estimates after IV, IM and PO administration of 50 mg in sheep.

		IV				IM				PO			
Parameter	Unit	Mean	SD	Median	Range	Mean	SD	Median	Range	Mean	SD	Median	Range
λz	1/h	0.39	0.036	0.38	0.34–0.45	0.42	0.05	0.41	0.37–0.50	/	/	/	/
T1/2 λz	h	1.8	0.16	1.82	1.54–2.02	1.66	0.19	1.69	1.40–1.88	/	/	/	/
Cmax	ng/ml	/	/	/	/	4332.83[c]	340.76	289	198–365	286[b]	64.74	289	(198–365)
Tmax	h	/	/	/	/	0.25	0	0.25	0.25	2	0	2	2
AUC (0-last)	ng h/ml	12518[b,c]	1037	12466	11096–14033	8889[a,c]	1086	9041	7519–10370.	563.44[a,b]	120.63	587.4	380.29–686.98
AUC (0-inf)	ng h/ml	12699[b]	1091	12641	11206–14305	9000[a]	1077	9144	7627–10462	/	/	/	/
CL	ml/Kg	121.5	9.89	120.5	110–137	/	/	/	/	/	/	/	/
Vss	ml/Kg h	241	26	238.5	213–282	/	/	/	/	/	/	/	/
MRT	h	2.13	0.09	2.13	2.02–2.27	2.185	0.054	2.19	2.11–2.27	/	/	/	/
F	%	/	/	/	/	70.73	3.49	72.19	70.50–63.83	5.94	1.09	5.77	4.50–7.82

Abbreviations: λz, terminal phase constant; $T_{1/2}$λz, terminal half-life; C_{max}, peak plasma concentration; T_{max}, time of peak concentration; $AUC_{(0\text{-last})}$, area under the plasma concentration-time curve from 0 to last time collected samples; $AUC_{(0\text{-inf})}$, area under the plasma concentration-time curve from 0 h to infinity; CL, clearance; Vss, 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.

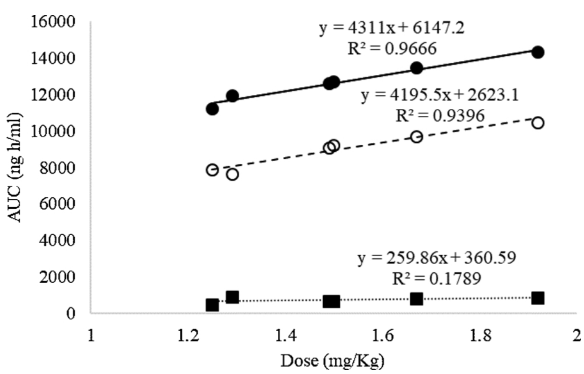

Fig. 2. Coefficient of determination (R^2) and estimated intercept and slop between weight-adjusted LSP doses and AUC (IV -●-; IM –o–; PO ··■··).

racemate sulpiride to stimulated ovulation/lactation and to evaluate the pharmacokinetics in veterinary species (Daels et al., 2000; Duchamp and Daels, 2002; Giorgi et al., 2013, 2015; Guillaume et al., 2003; Mari et al., 2009; Panzani et al., 2011). The pharmacokinetic profiles and estimates calculated in the present study, showed similarities with the profiles and estimates reported in goats (Łebkowska-Wieruszewska et al., 2019). Indeed, the Vss of LSP was similar to that reported in goats (goats: 244.65 ± 15.58 ml/h *vs* sheep: 241 ± 26 ml/h) (Łebkowska-Wieruszewska et al., 2019), but lower than those reported for sulpiride in the donkey and horse (Giorgi et al., 2013, 2015). Differently, CL resulted slightly lower than in goats (goats: 156.25 ± 8.09 ml Kg/h *vs* sheep: 121.5 ± 9.89 ml Kg/h) (Łebkowska-Wieruszewska et al., 2019) but higher than those reported for sulpiride in equine species (Giorgi et al., 2013, 2015). The differences in this latter parameter with equine species can be due to different cardiac output among the species (Toutain and Bousquet-Melou, 2004). However, sheep in this study had a similar body weight to the goats in the study of Łebkowska-Wieruszewska et al. (2019) and consequently similar cardiac output. The E, estimated according to allometric scaling, was low and next to that calculated in goats (Łebkowska-Wieruszewska et al., 2019). This suggests that sheep and goats have the same ability to eliminate LSP (Toutain and Bousquet-Melou, 2004). Then, it is assumable that the small difference in clearance may have a negligible clinical value in small ruminants. The T1/2λz value found in the present study was similar after both the injective administrations. However, it was somewhat higher than in goats (Łebkowska-Wieruszewska et al., 2019). This difference reflects the difference in CL values since T1/2λz is a hybrid parameter consisting of Vd and CL (Toutain and Bousquet-Melou, 2004). T1/2λz was lower than those reported for donkeys, horses, (Giorgi et al., 2013, 2015) and humans (Wiesel et al., 1980). These dissimilarities might result from species specific differences, different environmental conditions at the time of experiment, the period of time for blood collection or different sensitivities of the analytical method (Toutain and Bousquet-Melou, 2004). IM and PO F% were in line with those reported in goats (IM, sheep 70.7% *vs* goats 67.8%; PO, sheep 5.9% *vs* goats 4.9%) (Łebkowska-Wieruszewska et al., 2019). However, the AUC value calculated in the PO group might not be reliable because the calculation of the AUC was carried out with an inadequate number of observed data. The PO F% of sulpiride have been found higher in humans (23.4%) (Xu et al., 2015), in horses (20.4%) (Giorgi et al., 2013) and in donkeys (9.4%) (Giorgi et al., 2015). The pharmaceutical formulation developed specifically for humans, and/or the dilution of LSP in the rumen fluids, are factors that could have contributed to reduce the PO F% in sheep. A recent pharmacodynamic study showed that sulpiride significantly increased the rate of oestrus induction, ovulation, and lambing in sheep compared to the control group (Kumar Saxena et al., 2015). In this study animals were treated subcutaneously (SC) at the dosage of 0.6 mg/Kg twice a day till the onset of oestrus. In the present study an $AUC_{0\text{-}10}$ of 9152.31 ± 1087.23 ng h/ml with an average LSP plasma concentration value (Conc) of 915.23 ± 99.25 ng/ml was found. If it is assumed that LSP has the same F% after IM and SC administration and that LSP and sulpiride have the same intrinsic activity and affinity at the D2 receptors, from the following formula:

Effective concentration = Dose (0.6 mg/Kg) × Conc (915.23 ng/ml)/ Dose (1.51 mg/Kg)

the average concentration that produces the onset of oestrus may be estimated as 364.69 ± 10.92 ng/ml. It is important to notice that D2 antagonists have been found to follow concentration dependent pharmacodynamic (Bressolle et al., 1992; Giorgi et al., 2015).

In conclusion oral administration of LSP in sheep is not recommended due to both the low plasma concentration and bioavailability. IV and IM administration show comparable PK profiles. However, 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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References

Anonymous, 2009. Guideline on Validation of Bioanalytical Methods. EMEA/CHMP/EWP/192217/2009. .

Abecia, J.A., Forcada, F., González-Bulnes, A., 2012. Hormonal control of reproduction in small ruminants. Anim. Reprod. Sci. 130, 173–179. https://doi.org/10.1016/j.anireprosci.2012.01.011.

Bressolle, F., Brès, J., Fauré-Jeantis, A., 1992. Absolute bioavailability, rate of absorption, and dose proportionality of sulpiride in humans. J. Pharm. Sci. 81 (1), 26–32.

Daels, P.F., Fatone, S., Hansen, B.S., Concannon, P.W., 2000. Dopamine antagonist-induced reproductive function in anoestrous mares: gonadotrophin secretion and effects of environmental cues. J. Reprod. Fertil. Suppl. 56, 173–183.

Duchamp, G., Daels, P.F., 2002. Combined effect of sulpiride and light treatment on the onset of cyclicity in anestrous mares. Theriogenology 58, 599–602.

Fiorica, C., Palumbo, F.S., Pitarresia, G., Giorgi, M., Calascibetta, F., Giammona, G., 2015. In-situ forming gel-like depot of a polyaspartamide-polylactide copolymer for once a week administration of sulpiride. J. Pharm. Pharmacol. 67, 78–86. https://doi.org/10.1111/jphp.12323.

Giorgi, M., Ozdemir, M., Camillo, F., Panzani, D., 2013. Pharmacokinetics of sulpiride after intravenous, intramuscular, and oral single-dose administration in nurse mares. J. Equine Vet. Sci. 33, 533–538. https://doi.org/10.1016/j.jevs.2012.08.005.

Giorgi, M., Vullo, C., De Vito, V., Catone, G., Faillace, V., Laus, F., 2015. Pharmacokinetic evaluations of sulpiride after intravenous, intramuscular, and oral single-dose administration in jennies (*Equus asinus*). J. Equine Vet. Sci. 35, 13–18. https://doi.org/10.1016/j.jevs.2014.10.003.

Gong, C., Agbokponto, J.E., Yang, W., Simpemba, E., Zheng, X., Zhang, Q., Ding, L., 2014. Pharmacokinetics of levosulpiride after single and multiple intramuscular administrations in healthy Chinese volunteers. Acta Pharmacol. Sin. B 4 (5), 402–407. https://doi.org/10.1016/j.apsb.2014.06.001.

Guillaume, D., Chavatte-Palmer, P., Combarnous, Y., Duchamp, G., Martinat, N., Nagy, P., Deals, P.F., 2003. Induced lactation with a dopamine antagonist in mares: different responses between ovariectomized and intact mares. Reprod. Domest. Anim. 38, 394–400.

Hansel, W., Convey, E.M., 1993. Physiology of the estrous cycle. J. Anim. Sci. 57, 404–424.

Kumar Saxena, V.K., De, K., Kumar, D., Khursheed Naqvi, S.M., Krishnaswamy, N., Kumar Tiwari, A., 2015. Induction of ovulation in anestrus ewes using a dopamine receptor antagonist. Theriogenology 84, 1362–1366.

Łebkowska-Wieruszewska, B., Barsotti, G., Camillo, F., Rota, A., Panzani, D., Poapolathep, A., Lisowski, A., Giorgi, M., 2019. Pharmacokinetics of levosulpiride after single-dose administration in goats (*Capra hircus*) by different routes of administration. J. Vet. Pharmacol. Ther. 00, 1–7. https://doi.org/10.1111/jvp.12786.

Mari, G., Morganti, M., Merlo, B., Castagnetti, C., Parmeggiani, F., Govoni, N., Galeati, G., Tamanini, C., 2009. Administration of sulpiride or domperidone for advancing the first ovulation in deep anestrous mares. Theriogenology 71, 959–965. https://doi.org/10.1016/j.theriogenology.2008.11.001.

McCracken, J., Carlson, J.C., Glew, M.E., Goding, J.R., Baird, D.T., Green, K., Samuelsson, B., 1972. Prostaglandin F2 identified as a luteolytic hormone in sheep. Nat. New Biol. 238, 129–134.

Mucci, A., Nolfe, G., Maj, M., 1995. Levosulpiride: a review of its clinical use in psychiatry. Pharmacol. Res. 31, 95–101. https://doi.org/10.1016/10436618(95)800530.

Panzani, D., Zicchino, I., Taras, A., Marmorini, P., Crisci, A., Rota, A., Camillo, F., 2011. Clinical use of dopamine antagonist sulpiride to advance first ovulation in transitional mares. Theriogenology 75, 138–143. https://doi.org/10.1016/j.theriogenology.2010.07.019.

Rossi, F., Forgione, A., 1995. Pharmacotoxocological aspects of levosulpiride. Pharmacol. Res. 31, 81–94.

Rubianes, E., Menchaca, A., Carbajal, B., 2003. Response of the 1–5 day-aged ovine corpus luteum to prostaglandin F2alpha. Anim. Reprod. Sci. 78, 47–55.

Toutain, P.L., Bousquet-Melou, A., 2004. Plasma clearance. J. Vet. Pharmacol. Ther. 27, 415–425. https://doi.org/10.1111/j.1365-2885.2004.00605.x.

Walkden-Brown, S.W., Martin, G.B., Restall, B.J., 1999. Role of male female interaction in regulating reproduction in sheep and goats. J. Reprod. Fertil. 54, 243–257.

Wiesel, F.A., Alfredsson, G., Ehrnebo, M., Sedvall, G., 1980. The pharmacokinetics of intravenous and oral sulpiride in healthy human subjects. Eur. J. Clin. Pharmacol. 17, 385–391. https://doi.org/10.1007/BF00558453.

Xu, M., Zhou, Y., Ni, Y., He, X., Li, H., Sattar, H., et al., 2015. Tolerability and pharmacokinetic comparison of oral, intramuscular, and intravenous administration of levosulpiride after single and multiple dosing in healthy Chinese volunteers. Clin. Ther. 37, 2458–2467. https://doi.org/10.1016/j.clint hera.2015.08.024.