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Pharmacokinetics and bioavailability after intramuscular injection of the 5-HT$_{1A}$ serotonin agonist R-8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) in domestic goats (*Capra aegagrus hircus*)

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Abstract

To determine the bioavailability and pharmacokinetic properties of the serotonin 5-HT$_{1A}$ receptor agonist R-8-OH-DPAT in goats, and 0.1 mg kg^{-1} R-8-OH-DPAT hydrobromide was administered intramuscularly (i.m.) and intravenously (i.v.) to six goats in a two-phase cross-over design experiment. Venous blood samples were collected from the jugular vein 2, 5, 10, 15, 20, 30, 40 and 60 min following treatment and analysed by liquid chromatography tandem mass spectrometry. Bioavailability and pharmacokinetic parameters were determined by a one-compartment analysis. Mean bioavailability of R-8-OH-DPAT when injected i.m. was 66%. The mean volume of distribution in the central compartment was 1.47 L kg^{-1}. The mean plasma body clearance was 0.056 L kg^{-1} min^{-1}. All goats injected i.v. and two of six goats injected i.m. showed signs of serotonin toxicity. In conclusion, R-8-OH-DPAT is well absorbed following i.m. injection and the observed pharmacokinetics suggest that administration via dart is feasible. Administration of R-8-OH-DPAT hydrobromide, at a dosage of 0.1 mg kg^{-1}, resulted in the observation of clinical signs of serotonin toxicity in the goats. It is suggested that dosages for the clinical use of the compound should be lower in order to achieve the desired clinical effect without causing serotonin toxicity.

KEYWORDS
8-OH-DPAT, bioavailability, pharmacokinetics, serotonin agonist, wildlife immobilization

1 | INTRODUCTION

Serotonergic ligands, specifically 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), through its effects on the 5-HT$_{1A}$ receptors, alleviate opioid-induced hypoxia by reducing respiratory depression and ventilation perfusion mismatch (Kimura & Haji, 2014; Meyer, Fuller, & Mitchell, 2006; Richter, Manzke, Wilken, & Ponimaskin, 2003; Sahibzada, Ferreira, Wasserman, Taveira-DaSilva, & Gillis, 2000; Stettner, Zanella, Hilaire, & Dutschmann, 2008). Importantly,

8-OH-DPAT does not affect analgesia, catatonia or sedation caused by opioids (Guenther et al., 2009; Haigh, 1990; Manzke et al., 2003; McCrimmon & Alheid, 2003; Meyer et al., 2006). It is thought that the use of the R-enantiomer of 8-OH-DPAT may produce even more pronounced results in comparison to the previously-studied racemic form because of its high specificity at the 5-HT$_{1A}$ receptors (Hadrava, Blier, & De Montigny, 1996; Lejeune, Newman-Tancredi, Audinot, & Millan, 1997; Yu, Liu, Malmberg, et al., 1996). Two papers reported clinical respiratory changes following the intravenous (i.v.)

use of the racemic form of 8-OH-DPAT in goats at dosages of 0.1 and 0.5 mg kg^{-1}, respectively (Herman, O'Halloran, & Bisgard, 2001; Meyer et al., 2006). In order to immobilize wild herbivores, R-8-OH-DPAT would have to be administered together with the opioid by means of an intramuscular (i.m.) injection with a dart syringe.

Some literature on the pharmacokinetic data of 8-OH-DPAT in rats and marmosets exists (Kotani, Urushino, Natsutani, Ogi, & Ikeda, 2017; Yu & Lewander, 1997; Zuideveld, Treijtel, Van der Graaf, & Danhof, 2000) but there is currently no published literature available on the pharmacokinetics of 8-OH-DPAT in ungulates. Therefore, the pharmacokinetics and bioavailability after i.m. injection of this serotonin agonist had to be investigated in order to establish if it would be a feasible adjunct to an opioid-based i.m. immobilization mixture for wildlife.

The aim of this study was to characterize the pharmacokinetics and bioavailability of R-8-OH-DPAT hydrobromide in goats at a dosage of 0.1 mg kg^{-1} which equals 0.075 mg kg^{-1} of R-8-OH-DPAT.

2 | MATERIAL AND METHODS

This study was approved by the Murdoch University Animal Ethics Committee (R2922/17) and the animal ethics committee of Wildlife Pharmaceuticals (WPAEC-2016-DPATGOAT-09-C). Six healthy domestic female indigenous veld goats of between 4 and 5 years of age weighing between 29 and 43 kg (average 34 kg) were used for this project.

A two-way cross-over experiment was undertaken with a 14-day rest period.

Each goat was injected either i.v. or i.m. with 0.1 mg kg^{-1} R-8-OH-DPAT hydrobromide (Tocris Bioscience, Bristol, UK, catalogue number 1080), equivalent to approximately 0.075 mg kg^{-1} R-8-OH-DPAT (pubchem.ncbi.nlm.nih.gov). The compound was dissolved in sterile water to a concentration of 5 mg ml^{-1}. Intravenous injection was administered with a 21G 25.4 mm needle as a bolus into the jugular vein opposite to the catheterized jugular vein. After the i.v. injection, blood was collected back into the syringe and reinjected to ensure that the full dose of R-8-OH-DPAT was administered. The i.m. injection was given into the lateral femoral muscle group with a 21G 25.4 mm needle.

Blood was collected from an intravenous catheter that was inserted into the jugular vein. Serial blood samples were collected at 2, 5, 10, 15, 20, 30, 40 and 60 min following treatment. Within two hours after collection, the blood was centrifuged and serum decanted into cryovials and stored at −20°C until further analysis within 2 weeks after collection.

Laboratory analysis of the serum was performed by means of liquid chromatography tandem mass spectrometry (LC-MS/MS) by FDA Laboratories, Pretoria, South Africa similar to methods published (Kotani et al., 2017; Yu, Liu, Hacksell, & Lewander, 1996). The serum (20 µl) was diluted with methanol. The mixture was then centrifuged for protein precipitation. The supernatant was collected and subjected to the analysis against a reference standard prepared by dissolving R-8-OH-DPAT powder in methanol. Chromatographic separation was achieved using a Phenomenex Luna® 5 µm C18 150 × 2 mm analytical column maintained at 40°C. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) the gradient elution programme was as follows: 0–1 min, 90% A; 1–2 min, 90%–10% A; 2–5 min, 10% A; 5–6 min, 10%–90% A; 6–10 min, 90% A.

The LC-MS/MS system consisted of an Agilent 1200 high-pressure liquid chromatography system coupled to an AB Sciex triple quadrupole 3500 mass spectrometer. Positive electrospray ionization was used, and the analysis was performed in the multiple electron monitoring mode (MRM). The turbo ion spray source was set with the following settings: capillary voltage 5500 V, nebuliser gas (N$_2$) 10 (arbitrary units), curtain gas (N$_2$) 20 (arbitrary units) and source temperature of 400°C. Two MRM transitions were selected, the most intense (248.105 m/z:147.000 m/z) being used for quantification and the other (248.105 m/z:107.000 m/z) for confirmation.

The limit of quantification (LOQ) was set according to methods described by Shabir (2003) at 20 µg L^{-1}. The limit of detection (LOD) was estimated to be 5 µg L^{-1} as the lowest non-zero observation in the data set was 5.7 µg L^{-1}. The interassay variation was 12.53%, and the interassay variation was 10.35%.

Matrix-matched calibration curve and control samples were prepared by spiking on serum, collected from goats that had not been treated with the drug, at different concentrations. Serum concentrations of R-8-OH-DPAT were collectedby the use of peak area and slope-intercept from linear matrix-matched calibration curves.

Data management was conducted in Microsoft Excel 2016. Analysis and visualization of data and definition of models were performed in MATLAB 2015 (The Mathworks, Inc.). Coding for the study was developed based on the author's previous work (Sebbag, Thomasy, Woodward, Knych, & Maggs, 2016).

A typical data analysis scheme for the two-stage setting was used for pharmacokinetic modelling. For the compartmental analysis, after viewing the intravenous data on log$_{10}$ scale, a one-compartment model (Toutain & Bousquet-Melou, 2004a; Toutain & Bousquet-Mélou, 2004b) was chosen. The model for i.v. administration was implemented as the ordinary differential equation (ODE) (Equation 1), where DX$_1$/dt is the rate of change of drug mass in the central compartment, X$_1$ is the drug mass (µg) in the central compartment, V$_c$ is the central compartment volume of distribution (L kg^{-1}), and Cl$_p$ is the plasma clearance (L kg^{-1} min^{-1}).

$$\frac{dX1}{dt} = \left(\frac{X1}{Vc}\right) {}^*Clp \tag{1}$$

Numeric solutions of the ODE were collected for this model using the numeric differentiation formulas as implemented in MATLAB's "ode15s" (Shampine & Reichelt, 1997).

For the i.m. model (Equations 2 and 3), we defined first-order absorption, where X$_a$ is the drug mass in the absorption compartment (µg) and k$_a$ is the first-order absorption rate constant (min^{-1}).
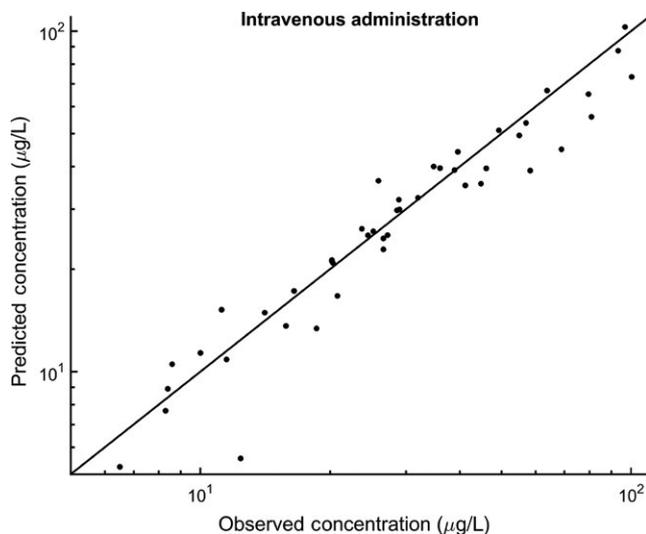
FIGURE 1 Observed versus predicted concentrations of R-8-OH-DPAT in serum of goats after i.v. injection of 0.1 mg kg^{-1} R-8-OH-DPAT hydrobromide. The solid line is the line of identity ($y = x$)

$$\frac{dX1}{dt} = -\left(\left(\frac{X1}{Vc}\right)*Clp\right) + (Xa*ka) \tag{2}$$

$$\frac{dXa}{dt} = -(Xa*ka) \tag{3}$$

This model includes i.m. bioavailability $F_{i.m.}$ (%), implemented in the ODE initial conditions. The model therefore includes four primary pharmacokinetic parameters to be estimated from the data; total plasma clearance (CL_P), volume of distribution of the central compartment (V_c), first-order absorption rate constant (k_a) and intramuscular bioavailability ($F_{i.m.}$). The error standard deviation (σ_ε^2) is also estimated in this model.

Parameter estimation was governed by the following principles:

1. all BLQ data points were included as scalar observations, as for the observations above the quantification limit (LOQ) (i.e. the LOQ was ignored).
2. the BLD data points were defined as censored observations (i.e., <5 µg L^{-1}).
3. a censored non-linear regression model was defined, and instead of minimizing the sum-of-squares of the weighted residuals, we implemented maximum likelihood estimation of the pharmacokinetic parameters, first for the i.v. phase only, then for the i.v. and i.m. phases simultaneously.

For step 3, the censored (Tobit) regression was implemented by direct definition of the log-likelihood function (Lynn, 2001), with optimization using the MATLAB functions "fmincon" (constrained function minimization), an implementation of an interior-point algorithm, and "patternsearch," an adaptive mesh algorithm. For the BLD, this censored regression method is equivalent to Beal's "method 3" (Beal, 2001). For the maximum likelihood estimation,

we assumed the distribution of residual errors was normal, with standard deviation as a parameter to be estimated (σ_ε^2). To accommodate heteroscedasticity, the residuals were weighted (1/Y). We estimated simultaneous parameters for the i.v. and i.m. phase for each subject, to improve the robustness of the i.m. parameter estimates. The goodness-of-fit of the models was assessed by plots of the observed and predicted concentrations vs time for each subject, and identity (observed vs predicted concentrations) and residuals plots across subjects.

Secondary parameters were estimated from the final models.

The area under the curve (AUC) was determined by numeric integration of the final models. The AUC$_{inf}$ was obtained as the improper integral (integration from time zero to infinity).

The elimination and absorption half-lives were obtained from Equation 4.

$$t\frac{1}{2} = \frac{\log_e 2}{k} \tag{4}$$

where k is the rate constant. As the model was parameterized using clearance, the elimination rate constant was obtained as clearance divided by the volume of distribution.

3 | RESULTS

The i.m. data series from one subject was excluded from analysis as it contained no useful information and was probably indicative of drug administration error (numerous BLD observations). The final analysed data set included 88 observations, from six i.v. subject-occasions and five i.m. subject-occasions.

Though the experiment was a conventional two-stage, crossover design, the data set was characterized by observations that were below the analytical method detection limit (BLD; n = 6/88), or below the quantification limit but above the detection limit (BLQ; n = 40/88).

The final compartmental model was an acceptable fit, to both the i.m. and the i.v. data. The goodness-of-fit visualization indicated that the fit of the i.v. model across subjects was similar to that of the i.m. model across subjects (Figures 1 and 2). For all the modelled subject-occasions, the quality of the model fit was similar; none stood out as being indicative of model failure.

Estimated primary and secondary parameters from the compartmental analysis are listed in Table 1 and Table 2, respectively.

All goats injected i.v. displayed clear clinical signs of serotonin toxicity (Boyer, 2005; Isbister & Buckley, 2005; Volpi-Abadie, Kaye, & Kaye, 2013), while two of five goats injected i.m. displayed mild signs of serotonin toxicity. The clinical behavioural effects of the R-8-OH-DPAT manifested within 2 min and 5 min after injection in goats that were injected i.v. and i.m., respectively. These clinical signs consisted of head shaking, chewing and licking, moderate to severe shivering of the entire body, vocalization, urination and hypersensitivity to external stimulation. All goats fully recovered within twenty minutes after onset of the clinical signs.

4 | DISCUSSION

The lower limit of quantification in this study coincided with biologically relevant drug concentrations. Because the number of BLQ observations was large, and more prevalent in the i.m. phase, the reported concentrations of the BLQ observations were used directly rather than deleting these observations from the data set. Despite widespread use in analytical method validation, quantification limits depend on arbitrary selection of an acceptable relative error limit, despite the fact that measurement error is present in all the data (Guo, Harel, & Little, 2010; Jelliffe, Schumitzky, Bayard, Fu, & Neely, 2015). Simply discarding the BLQ data would result in complete failure of the i.m. phase, even though it contains useful information. The presence of BLQ data requires careful consideration of appropriate methodology to limit bias in parameter estimation while retaining
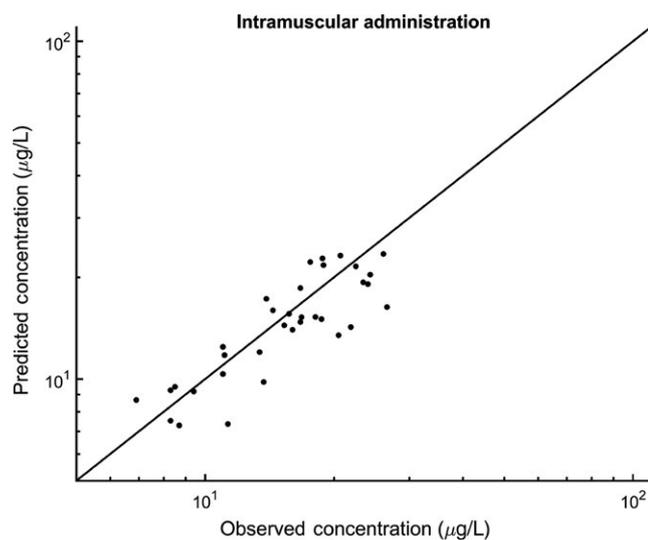

FIGURE 2 Observed versus predicted concentrations of R-8-OH-DPAT in serum of goats after i.m. injection of 0.1 mg kg^{-1} R-8-OH-DPAT hydrobromide. The solid line is the line of identity ($y = x$)

information (Jusko, 2012). Recent observations in population analysis demonstrate that simply ignoring the LOQ is a valid approach, where numeric values for the BLQ observations are available, and superior to deletion or substitution (Keizer et al., 2015). In this study, the BLQ observations were retained, combined with the popular maximum likelihood approach (Bergstrand & Karlsson, 2009) for the BLD observations. However, the precise impact of measurement error associated with BLQ observations on the parameter estimates in this study is not known, and there is a specific risk of inaccuracy in the estimate of bioavailability due to the greater number of BLQ observations in the i.m. phase. The interference of the LOQ with plasma concentrations achieved after a therapeutically relevant dose suggests that optimization of analytical methods is a key priority for further investigations of this drug.

Establishing bioavailability estimates for R-8-OH-DPAT administered via the i.m. route has significant practical value. This knowledge can be used to ascertain the practicality and a dose estimate for adding the serotonin agonist to an opioid-based immobilization dart mixture. Although there was a large variability (range 50% to 98%), the mean bioavailability was 66% after i.m. injection; therefore, R-8-OH-DPAT was well absorbed from the injection site. At the chosen concentration of 5 mg mL^{-1} R-8-OH-DPAT hydrobromide, dissolved in sterile water, the total drug volume per animal was low. This means that a dart preparation is feasible due to the low injection volume.

With an absorption half-life of six minutes, the highest serum concentrations of R-8-OH-DPAT were reached in all goats between 11 and 17.5 minutes after i.m. injection (Figures 3 and 4). The mean elimination half-life of R-8-OH-DPAT in goats was 18 min (CV = 17%) according to the compartmental analysis in our experiment. This result is similar to that in rats reported by Yu and Lewander (1997). These researchers showed that in rats the plasma half-life of R-OH-DPAT reached 27 min.

The use of serotonin agonists can be associated with serotonin toxicity, also called serotonin behavioural syndrome (Boyer, 2005; Isbister, Buckley, & Whyte, 2007; Volpi-Abadie et al., 2013).

TABLE 1 Estimated primary parameters from compartmental analysis for the six subjects

Subject ID	V_c	Cl_P	σ_e^2	k_a	$F_{i.m.}$
1	1.049	0.03991	0.2331	0.1013	0.6448
2	1.962	0.06594	0.4216	0.1436	0.9769
3	0.7193	0.03987	0.4762	0.07038	0.5007
4	1.416	0.05617	0.2171	0.23015	0.5376
5	1.7486	0.07031	0.2393	0.1427	0.6393
6	1.94845	0.06496	0.2680	*	*
MEAN	1.474	0.0562	0.3092	0.1376	0.6599
MEDIAN	1.582	0.0606	0.2536	0.1427	0.6393
CV%	34.489	23.91	35.83	43.69	28.5

Note. σ_e^2 Is the standard deviation of the residual error distribution; CV% is the coefficient of variation (STD/MEAN*100); Cl_P is the plasma body clearance (L kg^{-1} min^{-1}); $F_{i.m.}$ is the bioavailability after intramuscular administration (as proportion); k_a is the first-order absorption rate constant after intramuscular administration (min^{-1}); MEAN is the arithmetic mean. STD is the sample standard deviation; V_c is the central compartment volume of distribution (L kg^{-1}).
*Not estimated as the available i.m. data for this subject were excluded.

TABLE 2 Estimated secondary parameters from the compartmental analysis for the six subjects

Subject ID	T_{max}	C_{max}	$t_{1/2}$ A	$t_{1/2}$ E	AUC$_{inf}$ i.v.	AUC$_{inf}$ i.m.
1	17.48	27.03	6.841	18.23	1889.2	1220.7
2	15.20	47.35	4.827	20.62	1143.4	1119.2
3	17.97	15.61	9.848	12.51	1895.7	949.0
4	11.23	28.16	3.011	17.47	1342.5	723.0
5	14.36	29.38	4.857	17.24	1072.7	686.8
6	*	*	*	20.79	1160.8	*
MEAN	15.25	29.51	5.877	17.81	1417.4	939.7
MEDIAN	15.20	28.16	4.857	17.85	1251.6	949.0
CV%	17.77	38.63	44.25	16.94	26.71	25.08

Note. AUC $_{inf}$i.v. and AUC$_{inf}$i.m. are the predicted area under the curve extrapolated to infinity, for the i.v. and i.m. phase, respectively (μg hr L^{-1}); CV% is the coefficient of variation (sample standard deviation/MEAN*100); C_{max} is the predicted maximum serum concentration during the i.m. phase (μg L^{-1}); MEAN is the arithmetic mean. MEDIAN is the sample median; T_{max} is the predicted time of maximum plasma concentration for the i.m. phase (minutes); $t_{1/2}$ A is the absorption half-life (minutes); $t_{1/2}$ E is the elimination half-life (minutes).
*Not estimated as the available i.m. data for this subject were excluded.
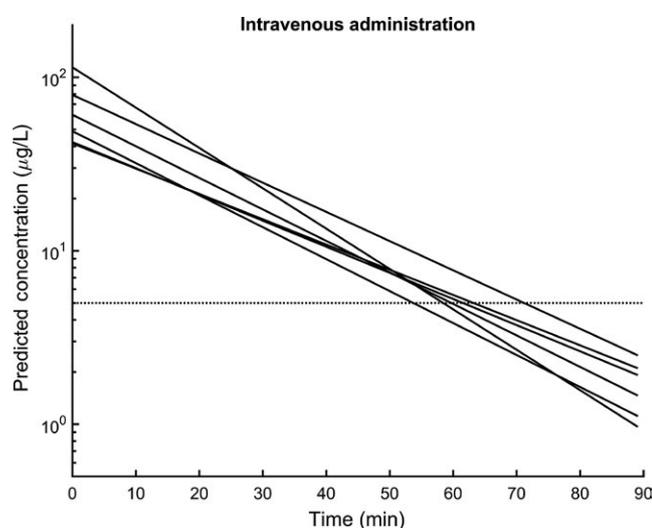
FIGURE 3 Predicted serum concentrations in goats after i.v. injection of 0.1 mg kg^{-1} R-8-OH-DPAT hydrobromide. The solid lines each represent predictions for one subject while the dotted line is the analytical detection limit (LOD; 5 μg L^{-1})
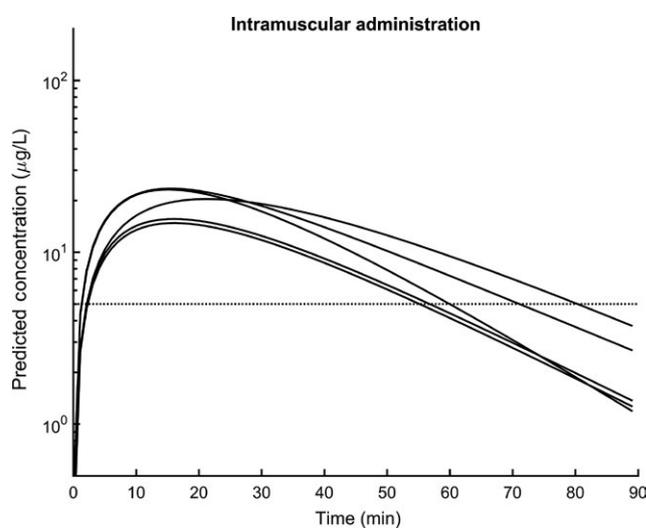
FIGURE 4 Predicted serum concentrations in goats after i.m. injection of 0.1 mg kg^{-1} R-8-OH-DPAT hydrobromide. The solid lines each represent predictions for one subject while the dotted line is the analytical detection limit (LOD; 5 μg L^{-1})

Serotonin toxicity has been described in humans and animals (Isbister & Buckley, 2005). The choice of dosage in the current experiment (0.1 mg kg^{-1} of R-8-OH-DPAT) was based on previous reports in goats (Herman et al., 2001; Meyer et al., 2006) in which up to 0.5 mg kg^{-1} 8-OH-DPAT was administered i.v. No adverse effects were reported, suggesting that the dose (0.1 mg kg^{-1}) in this experiment would be safe. It is postulated that the onset of serotonin toxicity in the experimental animals was associated with the use of the R-enantiomer of 8-OH-DPAT as opposed to the racemic mixture that was used in other studies. The R-enantiomer has been shown to have more specific action on the 5-HT$_{1A}$ receptors (Hadrava et al., 1996; Lejeune et al., 1997; Yu, Liu, Malmberg, et al., 1996) and the likely reason for the toxicity clinical signs described here.

5 | CONCLUSIONS

According to the pharmacokinetic analysis, R-8-OH-DPAT was readily absorbed with a mean bioavailability of 66% after i.m. injection and the observed pharmacokinetics suggest that administration via dart is feasible. Clinical relevance of the achieved concentrations is evidenced by clinical signs of serotonin toxicity observed after injection. Following the observation of clinical signs of serotonin toxicity in the goats at a dosage of R-8-OH-DPAT hydrobromide of 0.1 mg kg^{-1}, it is concluded that the dosage used should be lower in order to achieve the desired clinical effect without causing serotonin toxicity.

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