Pharmacokinetics of tramadol administered by intravenous and intramuscular routes to female dogs submitted to ovariohysterectomy

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Abstract

The objective of the present study was to implant a method using a sensitive and specific system, and validate the whole analytical method to obtain an efficient tool for analyses of tramadol in plasma dogs, and to evaluate the pharmacokinetics of tramadol following intravenous (i.v.) and intramuscular (i.m.) administration of this drug in females dogs submitted to castration. The pharmacokinetics of tramadol were examined following i.v. or i.m. tramadol administration to five female dogs in each group submitted to ovariohysterectomy (dosage=2 mg/kg). In relation to intravenous administration, the half-time for the distribution process ($t_{1/2d}$ = 0.18 ± 0.12 h); the total body clearance was 0.60 ± 0.50 L/h/kg, half-life of elimination ($t_{1/2b}$) was 1.24 ± 0.69 h. Statistically differences between parameters obtained after i.v. and i.m. was significant only to $AUC_{0-\infty}$: 3362.07 ± 1008 and 1604.55 ± 960.02 (ng.h/mL), respectively. The F was 48.00 ± 43.30 %. The assay for tramadol described has been demonstrated to meet all requirements for clinical PK studies. In particular, the method has satisfactory specificity, linearity, accuracy and precision range over the concentration examined.

Key words:
Tramadol.
Castration.
Dogs.
Pharmacokinetics.

Introduction

Tramadol hydrochloride, (1RS, 2RS)-[(dimethylamin) methyl]-1-(3-methoxyphenyl) cyclohexanol HCl, is a centrally acting opioid analgesic in wide spread human clinical use.[1] It is a synthetic analogue of codeine, but has a relatively low affinity for opiate receptors. Tramadol has been used for postoperative analgesia following orthopedic surgery and major gynecologic surgeries in addition to nonsurgical conditions in humans.[2,3,4,5]

Tramadol is well absorbed and extensively metabolized after oral administration in human beings, and its metabolites are excreted primarily in the urine.[6] Unchanged tramadol and a total of twenty four metabolites, consisting of sixteen phase I metabolites and eight conjugates (seven glucuronides, one sulfate), were isolated in the urine of dogs and rats.[7] Minimum effective plasma concentration in human beings for tramadol and O-desmethyltramadol, an active metabolite, have been reported to be 298 ± 171-590 ± 410 and 39.6 ± 29.5 – 84 ± 34 ng/mL, respectively in postoperative human patients.[4,8]

At "Veterinary Hospital of University of São Paulo", tramadol has been used as analgesic after ovariohysterectomy (castration) of female dogs. Therefore, there is a lack of pharmacokinetics data of tramadol in this animal specie by intramuscular (i.m.) route of administration. Thus, the objective of the present work was to: (i) implant a method using a sensitive and specific system, and validate the whole analytical method to obtain an efficient tool for analyses of tramadol in plasma dogs, and (ii) evaluate the pharmacokinetics (PKs) of tramadol following intravenous (i.v.) and

intramuscular (i.m.) administration of this drug in females dogs submitted to castration.

Material and Method

Ten adult mixed breed female dogs were enrolled in this study between March and December 2005. The mean age was 2.75 years old (range: 1 – 6 years) and the mean body weight was 28.89 kg (range: 15 – 55.7 kg). All animals were considered healthy, based on physical examination, complete blood count, plasma biochemistry profile and urinalysis. All procedures related to this study were performed in accordance with The Institutional Animal Care and Use Committee at Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo.

The dogs were anaesthetized prior to and during ovariohysterectomy surgery with acepromazine, propofol and isoflurane. After the last stitch, each the dogs were randomly allocated in one of the two groups and administered a single dose of tramadol HCl commercial injection (Cristália, Brazil) (2 mg of tramadol per kg of body weight), i.v. via jugular vein or i.m. injected deep into semimembraneous muscle.

A jugular catheter was placed in the right jugular vein prior to surgery. Blood samples, 10 mL per sample, were collected in tubes containing sodium heparin as anticoagulant and centrifuged at 2000 g for 10 min. The plasma was decanted, labeled, frozen at - 80 °C until the assays were performed within 60 days of collection. This period is inferior to that established by Gan et al. [9] which is stable for more than 1 year when stored at - 20 °C. Samples were collected immediately before tramadol administration (0) and at 10, 20, 30, 45 minutes and 1.00, 1.50, 2.00, 3.00, 4.00, 5.00, 6.00 hours after tramadol administration.

Stock solutions of tramadol were prepared monthly by dissolving 11.38 mg of tramadol hydrochloride (Sigma®, Germany) in 100 mL of methanol (100 μg/mL) and kept stored at 4°C. Standard curve for plasma analysis were prepared by fortifying pooled fresh canine plasma with stock solution of tramadol hydrochloride to produce a concentration range from 10 to 2000 ng/mL. The fortified calibration samples were processed and prepared exactly as described bellow for the incurred plasma samples. Tramadol concentrations for the calibration curve were: 10, 50, 125, 250, 500, 1000 and 2000 ng/mL. These working solutions were made by further dilution of the stock solutions in methanol and they were prepared fresh daily. Deionized water was produced by a Milli-Q Millipore Water System (Millipore, MA, USA).

Intra-assay precision and accuracy were determined by measuring five replicates of each of three standard concentration (100, 750 and 1500 ng/mL) prepared in fresh dog plasma and then stored. Interassay precision and accuracy were estimated by assaying three plasma concentrations on four different days. Recovery was estimated by comparing the slope of the standard curves for extract plasma with that for the corresponding unextracted standards.

Plasma concentrations of tramadol were analyzed by high-performance liquid chromatography (HPLC model LC-10AD with UV-VIS spectrophotometric detector model SPD-10AV, Shimadzu, Analytical Instruments Division, Kyoto, Japan) at Laboratório de Farmacologia from Departamento de Patologia da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo. The HPLC method was based on previously published method [9,10,11] with modifications made to improve the efficiency of the method. For the analyses, frozen dog plasma samples were left on the bench to thaw naturally and were vortexed prior to their use. Plasma extraction was accomplished with liquid-liquid extraction. Briefly, to the plasma was added 5 drops of 0.1 M sodium hydroxide prior to the extraction. The solution was thoroughly vortexed. Then, 4 mL of ethyl acetate: hexane (1:4) (HPLC grade, Merck, Darmstadt, Germany) was added into the plasma and vortexed for 1.5 min. Afterward, it was subjected to centrifugation

at 3500 g for 15 min. The organic layer was transferred into Cahan's tubes. The tubes were then passed through a stream of nitrogen for drying (15 min) and 50 ml of the mobile phase was added for reconstitution before injection to the HPLC system. The analytical column was a RP-18 with particle size of 5 μm maintained at 55°C (Shimadzu, Maryland, USA). The mobile phase was constituted of 70% 0.01 M phosphate buffer adjusted to a pH of 5.9 with phosphoric acid (both of analytical reagent-grade from Merck, Darmstadt, Germany) with 0.1% triethylamine and 30% acetonitrile (HPLC grade, Merck Darmstadt, Germany). The UV detector was set to an excitation wavelength of 218 nm. The volume of each injection was 10 μL. Retention time for tramadol was 9.13 min and rate flow 1.2 mL/min.

Data analysis

Individual tramadol concentration vs. time curves was analyzed by non linear least square regression analysis using GraphPad Prism (1999). Choice of appropriate pharmacokinetic model was prepared on the basis of the lowest weighted sum of squares and lowest Akaikes's information criterion value for the individual data [12].

Following i.v. administration the final pharmacokinetic model fit the data was a two-compartment open model with first-order elimination from the central compartment in all the animals (Eqn 1):

$$C(t) = A\, e^{(-\alpha t)} + B\, e^{(\beta t)} \quad (1)$$

where C(t) (ng/mL) represents tramadol plasma concentration at time t; A and B (ng/mL) are the concentration extrapolated to time 0 of the first and second phase of tramadol plasma and α and β (1/h) are the distribution and elimination slopes, respectively.

Plasma tramadol distribution and elimination half-lives, $t_{1/2(d)}$ and $t_{1/2\beta}$, respectively, were calculated by Eqns 2 and 3:

$$t_{1/2(d)} = 0.693/\alpha \quad (2)$$

$$t_{1/2\beta} = 0.693/\beta \quad (3)$$

Area under the plasma curve from 0 to infinity ($AUC_{0-\infty}$) and area under the first moment curve from 0 to infinity ($AUMC_{0-\infty}$) were calculated by the linear trapezoidal method with extrapolation to infinity. The extrapolated area was estimated by Eqn 4 and 5:

$$AUC_{last-\infty} = C_{last}/\beta \quad (4)$$

$$AUMC_{last-\infty} = (t_{last} \times C_{last}/\beta) + C_{last}/k_{el}^2 \quad (5)$$

In which C_{last} is the last measured concentration, t is the time of C_{last}, and k_{el} was the elimination constant.

Total body clearance (Cl_T) was determined by Eqn (6):

$$Cl_T = dose/\ AUC_{0-\infty} \quad (6)$$

Mean residence time (MRT) was determined by Eqn (7):

$$MRT = AUC_{0-\infty}/\ AUMC_{0-\infty} \quad (7)$$

The apparent volume of distribution area was calculated by Eqn 8:

$$V_{d(area)} = dose/\ (AUC_{0-\infty} \times \beta) \quad (8)$$

The volume of central compartment was calculated by Eqn 9:

$$V_1 = dose/\ Cp_0 \quad (9)$$

Where $Cp_0 = A+B$

The micro constants were calculated by Eqn 10-12:

$$k_{21} = (\alpha B + A\beta)/A + B \quad (10)$$

$$k_{el} = \alpha\beta/k_{21} \quad (11)$$

$$k_{12} = \alpha + \beta - k_{21} - kel \quad (12)$$

Compartmental analysis parameters

were calculated from equations published elsewhere.[13] They are presented in table 1.

Tramadol plasma disposition curves after i.m. administration, were analyzed following the same procedure as used for i.v. analysis. Peak concentrations (C_{max}) of tramadol in blood and the time of peak concentration (Tmax) were obtained directly from the experimental data without interpolation. Systemic bioavailability (F) of tramadol was calculated from noncompartmental parameters using Eqn (13):

$$F = (AUC_{0-\infty\ i.m.} / AUC_{0-\infty\ i.v.}) \times 100 \quad (13)$$

Variance analysis (ANOVA) followed by Unpaired t test with Welch correction was used to analyze data from pharmacokinetic parameters. The results were presented as the mean with their standard deviation. All analyses were realized using the software GraphPad Instat [14] and the figure by using GraphPad Prism [15]. In all experiments, $P<0.05$ was the criterion for statistical significance.

Results

The linear concentration range for tramadol analysis was 10 to > 2000 ng/mL (n=7) ($r^2 > 0.999$). The limit of detection and quantification were found to be, respectively, 10 ng/mL and 50 ng/mL. The recoveries at 100, 750 and 1500 ng/mL were 87.5%, 87.7% and 86.5%, respectively. Intra-

Table 1 - Mean ± SD values for tramadol pharmacokinetic variables following intravenous (i.v.) and intramuscular (2 mg/kg) tramadol HCl administration to five adult female dogs, in each group, after ovariohysterectomy. Plasma concentration of tramadol was measured by high-performance liquid chromatography (HPLC)

Variable	i.v. route (mean ± SD)	i.m. route (mean ± SD)
α (1/h)	3.78 ± 2.21	NA
β (1/h)	0.56 ± 0.36	NA
A (ng/mL)	4800.00 ± 2000.00	NA
$AUC_{0\rightarrow\infty}$ (ng.h/mL)	3362.07 ± 1008.60*	1604.55 ± 960.02
$AUMC_{0\rightarrow\infty}$ (ng.h.h/mL)	3621.39 ± 2107.09	4300.83 ± 2627.00
B (ng/mL)	1200.00 ± 586.00	NA
C_0 (ng/mL)	6000.00 ± 3600.12	NA
Cl_T (L/h/kg)	0.60 ± 0.50	NA
Cl_T/F(L/h/kg)	NA	0.59 ± 0.38
C_{max} (ng/mL)	NA	625.50 ± 24.99
F (%)	NA	48.00 ± 43.30
ka	NA	0.64 ± 0.41
k_{12} (1/h)	1.38 ± 0.78	NA
k_{21} (1/h)	1.20 ± 0.65	NA
kel (1/h)	1.77 ± 0.49	NA
MAT (h)	NA	1.60 ± 0.97
MRT (h)	1.08 ± 0.63	2.70 ± 1.50
$t_{1/2\beta}$ (h)	1.24 ± 0.69	1.82 ± 1.01
$t_{1/2abs}$ (h)	NA	1.08 ± 0.62
$t_{1/2d}$ (h)	0.18 ± 0.12	NA
T_{max} (h)	NA	0.75 ± 0.25
$V_{d\ (área)}$ (L/kg)	1.06 ± 0.53	NA
V_{d1} (L/kg)	0.33 ± 0.21	NA

*Difference from i.v. and i.m. groups statistically significant. P<0.05.

Abbreviations: NA. not applicable; α = distribution slope; β = elimination slope; A = intercept for the distribution phase; $AUC_{0-\infty}$ = under the curve from time 0 to infinity; $AUMC_{0-\infty}$ = area under the first moment curve from 0 to infinity; B = intercept for the elim phase; C_0 = concentration at time 0; Cl_T = total body clearance; C_{max} = peak plasma concentration; F= systemic bioavailability; ka absorption; k_{12} = rate of movement from compartment 1 to 2; k_{21} = rate of movement from compartment 2 to 1; kel = rate of elimin MAT = mean absorption time; MRT = mean resident time; $t_{1/2\beta}$ = elimination half-life; $t_{1/2abs}$ = absorption half-life; $t_{1/2d}$ = distributi life; T_{max} = time of peak concentration; $Vd_{(area)}$ = apparent volume of distribution of the area; Vd_1 = apparent volume of the central compartment central.

and inter-day precision values for quality control samples were 2.2-3.2 and 2.8-3.3% coefficient of variation (CV), respectively. In terms of stability, no significant degradation of tramadol was observed under any of the storage conditions evaluated. There were no interfering peaks from control plasma matrix, hemolyzed or not, and the presence of acepromazine, propofol and isoflurane in the plasma. Mean retention time for tramadol was 9.13 min (Figure 1).

No adverse effects were noted after i.v. or i.m. administration of tramadol HCl at 2.0 mg/kg. All twelve dogs appeared mildly sedated after administration. Blood levels of tramadol administrated by i.v. and i.m are presented in figure 2 and the PK data are presented in table 1.

Blood samples taken from all evaluated animals before tramadol administration were found to contain no measurable levels of this drug. On the other hand, they presented high tramadol levels after dosage. By i.m. the highest tramadol concentration occurred at 0.75 ± 0.25 h (625.50 ± 24.99 ng/mL). In both routes these levels were measurable until 6 hours after tramadol administration. Tramadol levels were significantly higher by i.v. than i.m at all time evaluated.

A two-compartment model best fit the plasma concentrations after intravenous tramadol in all dogs. A one-compartment model with first-order input was fit to the plasma tramadol concentrations following i.m administration.

In relation to intravenous administration, tramadol serum concentration rapidly decreased during the first hour postadministration, as reflected by the half-time for the distribution process ($t_{1/2d}$ = 0.18 ± 0.12 h). Distribution was wide, with a Vd_1 of 0.33 ± 0.21 L/kg and a V_{area} of 1.06 ± 0.53 L/kg. The k_{12}/k_{21} ratio was 1.15 ± 0.58, indicating that the drug is returning rapidly from distribution sites for elimination from the body. Total body clearance was relatively rapidly (0.60 ± 0.50 L/h/kg). Half-life of

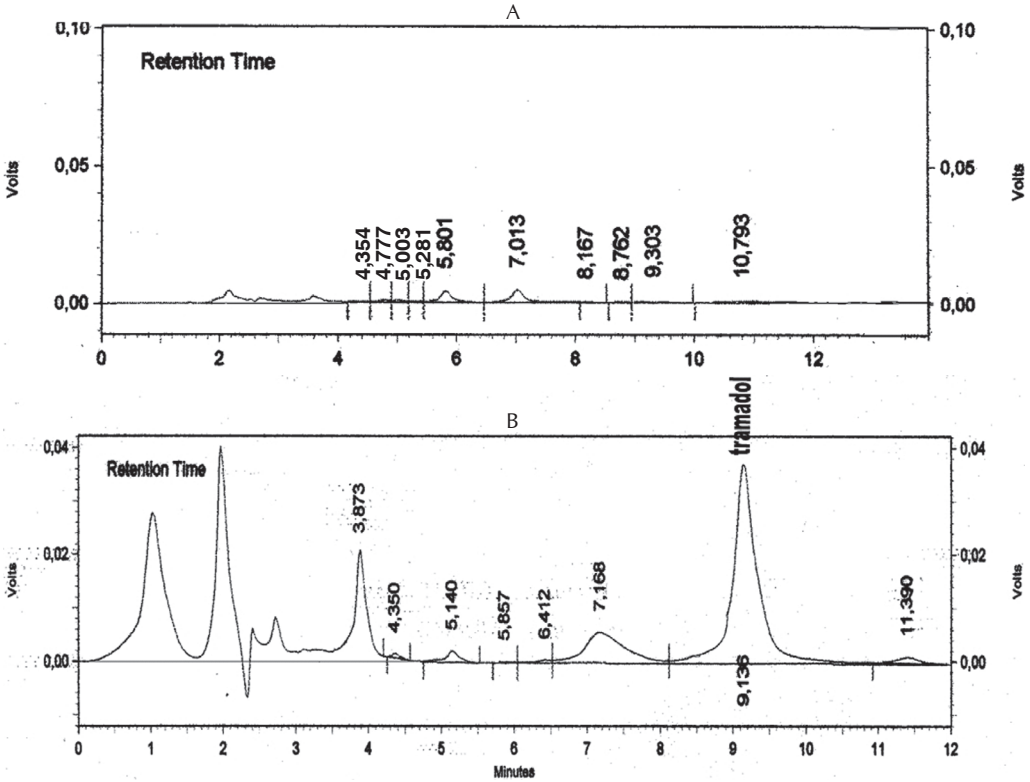

Figure 1 - Representative chromatograms obtained from (A) tramadol free dog plasma and (B) dog plasma from the experimental group that received 2 mg/kg/dose of tramadol by i.v. after ovariohysterectomy

elimination ($t_{1/2b}$) was 1.24 ± 0.69 h and a MRT of 1.08 ± 0.63.

On the other hand, calculated parameters in relation to i.m. administration showed the same median value to the total body clearance (0.59 ± 0.38 L/h/kg); the i.m. absorption was rapid as reflected by the T_{max} (0.75 ± 0.25 h) and $t_{1/2abs}$ (1.08 ± 0.62 h). Moreover, statistically differences between parameters obtained after i.v. and

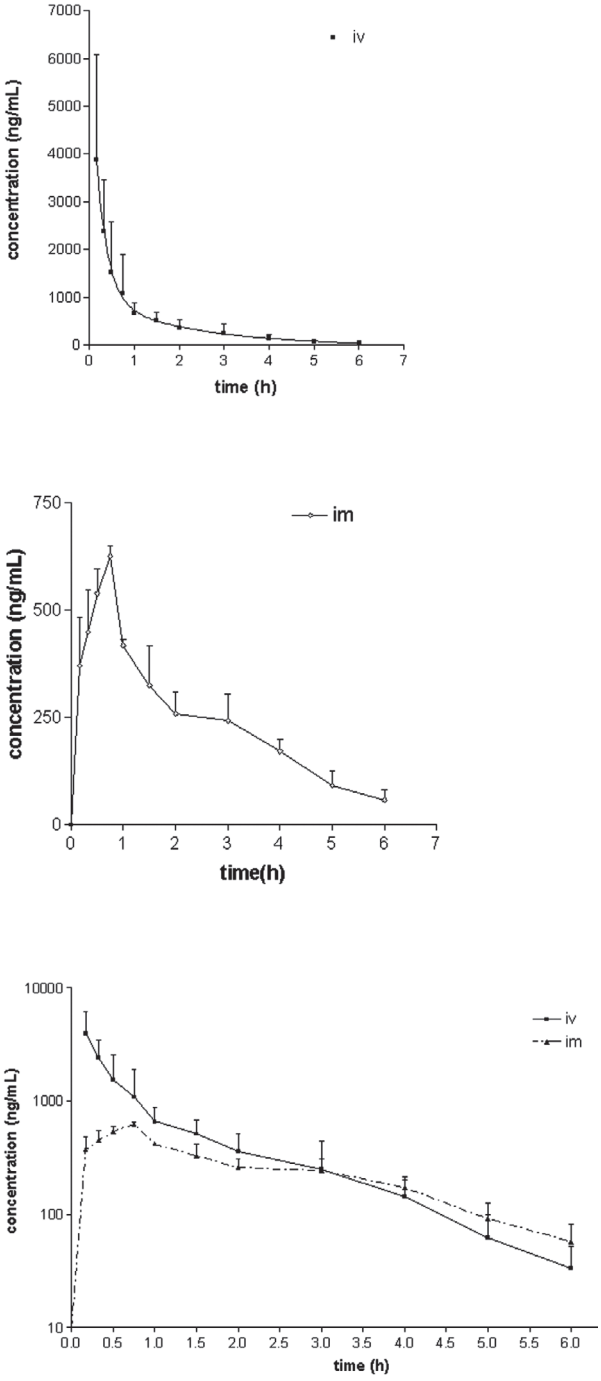

Figure 2 - Plasma concentration (ng/mL) profiles as measured by HPLC in adult female dogs after a single i.v. or i.m. administration of tramadol. Dosage = 2 mg/kg

i.m. was significant only in the $AUC_{0?\ \infty}$: 3362.07 ± 1008.60 and 1604.55 ± 960.02 (ng.h/mL), respectively. The F was 48.00 ± 43.30 %.

Discussion/Conclusion

Our study verified if the HPLC method previously published [9] to measure tramadol in human plasma was also appropriate to the dog plasma because this technique employees equipment and reagents available in our laboratory. The parameters analyzed showed that it is an effective technique with the advantages of being rapid, easy to perform and inexpensive.

This method has been successfully applied to the analyses of samples for a PK study in the present experiment which consisted of twelve dogs submitted to anesthesia with propofol, acepromazine and isoflurane and then submitted to castration. The dose of tramadol administered in the present investigation (2.0 mg/kg), in the end of the surgical procedure, was chosen taking into account previous studies [16] in which this dose was verified not to produce the typical adverse effects reported for tramadol and also because this dose generates detectable blood concentrations of the compound in treated animals.

Early studies in 1999 [16] demonstrated the analgesic effects of single-dose intramuscular tramadol 50-100 mg in human. Several studies have confirmed that repeated intramuscular administration of tramadol can provide effective and well tolerated postoperative analgesia comparable to that obtained with morphine, pentazoine and ketorolac.[17] In this way, we elected the i.m and i.v. routes as they are the main routes of drug administration after surgical procedures and also because there isn't any study comparing these two routes in dogs that received tramadol.

The tramadol plasma concentration vs time data after intravenous administration were best fitted to a two-compartment open model. This conclusion is in agreement with that found in previous studies of tramadol carried out in dogs.[16] An open one-compartment model with first-order absorption best fitted the data obtained after intramuscular administration of tramadol to female dogs.

Mean residence time (MRT) reflects the difference in persistence of the drug in the body after intravenous and intramuscular administration. The prolonged MRT after intramuscular administration compared to the intravenous administration, the clearances being similar, was due to the influence of the absorption phase. Similar results have been reported in dogs that received tramadol per os and intravenous.[16]

It was estimated that about 2% of the absorbed tramadol is excreted unchanged in the urine of dogs and more than 24 metabolites are excreted, which are almost completely eliminated through the kidneys.[7] In healthy humans, the average elimination half-life of tramadol was estimated at 6h, whereas in patients with renal insufficiency the dose must be adjusted according to the clearance renal values.[17] Assuming that the slower the elimination of this substance, the longer the time it will remain in the body, it could be inferred that both i.v. and i.m. dosing should expose the dogs lesser to tramadol than in human beings, as in the present study the elimination half-life of tramadol was 1.24 ± 0.69 and 1.82 ± 1.01, by i.v. and i.m., respectively.

The absorption process was rapid with a T_{max} range: 0.50 - 1.00 h and corroborated by the absorption rate constant (ka) and $t_{1/2abs}$. The C_{max} range: 650.49 – 600.51 ng/mL and the corresponding results after i.m. in humans was $C_{(max)}$ = 166 (1.24) ng/ml [6], which was inferior what could be in part be explained by the dose administered to human which was < 1 mg/kg. The F of the drug after i.m. administration at 2 mg/kg b.w. was 48.00 ± 43.30, in the present study. The low F values indicate that the drug was not completely absorbed from i.m. site injection in dogs. In contrast to its pharmacokinetics in humans, Ground et al.[6] and Lintz, Beier and Gerloff[18] founded 92.9 - 105.4%. Thus,

the results reflect the different kinetics between human and dogs

The volume of distribution area for tramadol was 1.06 ± 0.53 and volume of distribution of central compartment was 0.33 ± 0.21 L/kg, which is consistent with a high tissue affinity. On the other hand, extrapolation of this data should be viewed with caution when considering multidose studies as it has been well determined that many differences exist in biochemical, morphological and functional changes between single and prolonged exposure to drugs.[13]

In summary, taken as a whole, the present data strongly support that exposition to tramadol was able to raise the levels in the plasma of dogs submitted to castration. The assay for tramadol described has been demonstrated to meet all requirements for clinical PK studies. In particular, the method has satisfactory specificity, linearity, accuracy and precision range over the concentration examined. The results from i.v. and i.m. administration of tramadol reported here provided the PK information for the design of future studies of analgesic efficacy in dogs.

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Farmacocinética do tramadol administrado pela via intravenosa e intramuscular em cadelas submetidas a ovário - salpingo - histerectomia

Resumo

O objetivo do presente estudo foi de implantar um método sensível e específico, e validar toda a metodologia para obter uma ferramenta eficiente para a análise do tramadol em plasma de cadelas, e avaliar a farmacocinética do tramadol após a administração do mesmo pelas vias i.v. e i.m. em cadelas submetidas à castração. A farmacocinética do tramadol foi examinada após a administração do tramadol por ambas as vias, em cinco cadelas em cada grupo submetidas à ovário histerectomia (dose = 2 mg/kg). Em relação à administração intravenosa, a meia-vida de eliminação ($t_{1/2b}$) foi de 1,24 ± 0,69 h. Encontrou-se diferenças significantes somente nos parâmetros $AUC_{0-\infty}$: 3362,07 ± 1008 and 1604,55 ± 960.02 (ng.h/mL), pelas vias i.v. e i.m. respectivamente. O F foi de 48,00 ± 43,30 %. O estudo descrito neste artigo demonstrou atingir todas as exigências para os estudos clínicos em farmacocinética. Especificamente, o método apresentou especificidade, linearidade, exatidão e precisão satisfatórias no intervalo de concentrações examinadas.

Palavras-chave:
Tramadol.
Castração.
Cães.
Farmacocinética.

References

1 RAFFA, R. B. et al. Opioid and non opioid components independently contribute to the mechanisms of action of tramadol, an atypical opioid analgesic**. Journal of Pharmacology and Experimental Therapeutics**, v. 260, p. 275-285, 1992.

2 ELHAKIM, M. et al. Analgesic and antacid properties of i.m. tramadol given before Caesarian section under general anesthesia. **British Journal of Anaesthesia**, v. 95, p. 811-815, 2005.

3 GROUND, S. et al. Analgesic efficacy and safety of tramadol enantiomers in comparison with the racemate: a randomized, double-blind study with gynecological patients using intravenous patient-controlled analgesia. **Pain**, v. 62, p. 313-320, 1995.

4 LEHMANN, K. A.; SCHROEDER-BARK, U.; HORRICHS-HAERMEYER, G. Postoperative patient-controlled analgesia with tramadol: analgesic efficacy and minimum effective concentrations. **Clinical Journal of Pain**, v. 6, p. 212-220, 1990.

5 TUNCER, S. et al. Adding ketoprofen to intravenous

patient-controlled analgesia with after major gynecological cancer surgery: a double blind, randomized, placebo-controlled clinical trial. **European Journal of Gynecological Oncology**, v. 24, p. 181, 2003.

6 GROUND, S. et al. Serum concentration of tramadol enantiomers during patient-controlled analgesia. **British Journal of Clinical Pharmacology**, v. 48, p. 254-257, 1999.

7 WU, W. N. et al. Metabolism of the analgesic drug, tramadol hydrochloride, in rat and dog. **Xenobiotica**, v. 31, p. 423-441, 2001.

8 LINTZ, W. et al. Metabolismus von Tramadol bei Mensch and Tier. **Arzneim Forsch/Drug Research**, v. 31, p. 1931-1943, 1981.

9 GAN, S. H. et al. Method development and validation of a high-performance liquid chromatographic method for tramadol in human plasma using liquid-liquid extraction. **Journal of Chromatography B**, v. 772, p. 123-129, 2002.

10 GU, Y.; FAWCETT, J. P. Improved HPLC method for the simultaneous determination of tramadol and o-desmethyltramadol in human plasma. **Journal of Chromatography B**, v. 821, p. 240-243, 2005.

11 NOBILIS, M. et al. High-performance liquid chromatographic determination of tramadol and its o-desmethylated metabolite in blood plasma application to a bioequivalence study in humans. **Journal of Chromatography A**, v. 949, p. 1-22, 2002.

12 YAMAOKA, K.; TANIGAWARA, Y.; NAKAGAWA, T. T. Application of Akaikes's information criterion (AIC) in the evaluation of linear pharmacokinetic equations. **Journal of Pharmacokinetics and Biopharmaceutics**, v. 6, p. 165-175, 1978.

13 SHARGEL, L.; YU, A. B. C. **Applied biopharmaceuticals and pharmacokinetics**. New York: McGraw-Hill, 2005. 892 p.

14 GraphPad InStat. **Instat guide to choosing and interpreting statistical tests.** Version 3.00. San Diego, 1998.

15 GraphPad Prism. **GraphPad software**. Version 3.00. San Diego, 1999.

16 KUKANICH, B.; PAPICH, M. G. Pharmacokinetics of tramadol and the metabolite o-desmethyltramadol in dogs. **Journal of Veterinary Pharmacology and Therapeutics**, v. 27, p. 239-246, 2004.

17 GROUND, S.; SABLOTZKI, A. Clinical Pharmacology of tramadol. **Clinical Pharmacokinetics**, v. 43, p. 879-923, 2004.

18 LINTZ, W.; BEIER, H.; GERLOFF, J. Bioavailability of tramadol after i.m. injection in comparison to i.v. infusion. **International Journal of Clinical Pharmacology and Therapeutics**, v. 37, p. 175-183, 1999.

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1 - Limitar-se ao máximo de dez páginas digitadas, dentro da estrutura do item cinco, não sendo contadas as páginas onde constem tabelas e ilustrações. **2 -** Ser escrito em língua portuguesa ou em língua inglesa. **3 -** Usar somente nomenclaturas oficiais e abreviaturas consagradas, não empregando abreviaturas no título do artigo. **4 –** Ser estruturado dentro dos seguintes itens: a) Introdução; b) Materiais e Métodos; c) Resultados; d) Discussão; e) Conclusões; f) Referências; g) Resumo/Palavras-chave; Abstract/Key-words. **5 -** Apresentar, obrigatoriamente, dois resumos, nos idiomas inglês e português, não devendo ultrapassar 250 (duzentas e cinqüenta) palavras, seguidos das palavras-chave, limitadas a cinco, que correspondem a palavras ou expressões que identificam o conteúdo do artigo.

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Artigos de revisão

Só poderão ser publicados por especialistas de renome a convite da Comissão Editorial. Não devem ser subdivididos em seções separadas (Introdução, Materiais e Métodos etc.), mas devem apresentar, obrigatoriamente, dois resumos, com palavras-chave, conforme descrito na apresentação de Artigo completo, além de referências.

Apresentação dos trabalhos

1 - Digitação: original em CD, devidamente identificado com o título do artigo e nome do(s) autor(es) e três cópias impressas, inclusive suas tabelas e referências; deve ser digitado, obrigatoriamente, em formato A4 (21,0 x 29,7cm), espaço duplo, em uma só face de papel, margens de 2,5cm, fonte Times New Roman tamanho 10 e numeração consecutiva das páginas. Ilustrações e legendas devem ser relacionadas em folhas separadas. O texto dos artigos deve ser apresentado utilizando-se o editor de texto Microsoft Word. **2 - Página de rosto**: elemento obrigatório, onde deve conter o título do artigo, nome(s) do(s) autor(es) e instituição de origem. **Observar que unicamente nesta página conste a identificação dos autores, para o devido sigilo e imparcialidade.** No rodapé da página deve-se mencionar o endereço completo (inclusive e-mail) do autor para correspondência. Se o artigo for subvencionado, mencionar a instituição que o patrocinou, assim como os agradecimentos; **3 - Tabelas**: devem ser numeradas em algarismos arábicos e encabeçadas pelo título, seguido de local e data. Na montagem das tabelas seguir: IBGE. **Normas de apresentação tabular**. 3. ed. Rio de Janeiro: IBGE, 1993. 61 p. O limite de tabelas por trabalho é de cinco. Em casos excepcionais, conhecida a opinião da Comissão Editorial, este número poderá ser ultrapassado. No texto devem ser indicadas pela palavra Tabela (por extenso). **4 - Ilustrações** (fotografias, gráficos, quadros, desenhos ou esquemas): devem ser numeradas consecutivamente com algarismos arábicos e citadas como figuras no texto. As fotografias devem ser identificadas somente com o título do artigo, além de conter no verso a indicação de seu correto posicionamento. Fotos fornecidas em papel fotográfico devem ter ótima resolução, em CD com a extensão .TIF e resolução mínima de 300 dpi's. As legendas de ilustrações coloridas devem estar referenciadas somente por setas, símbolos e pontos quando publicadas em preto e branco. Gráficos, desenhos ou esquemas devem ser fornecidos no CD, impressos em folha à parte identificada somente com o título do artigo, além das respectivas legendas. Todas as ilustrações devem ser fornecidas em três vias. Os gráficos devem trazer sempre os valores numéricos que lhes deram origem. Desenhos e esquemas devem apresentar boa qualidade técnica e artística. Aceitar-se-á um número máximo de nove ilustrações por artigo, sugerindo-se a seguinte distribuição: três fotografias, três gráficos e três desenhos/esquemas. Acima deste limite, as despesas com reprodução correrão por conta do autor. Ilustrações coloridas, independentemente do número, serão cobradas. No texto devem ser indicadas pela palavra Figura (por extenso). Indicar junto ao título da ilustração o local e data. **5 - Referências**: devem ser numeradas, ao final do artigo, de forma consecutiva de acordo com a ordem em que forem sendo citadas no texto. Os títulos de periódicos devem ser mencionados de maneira uniforme, de preferência todos por extenso. As referências seguem a normalização da Associação Brasileira de Normas Técnicas (ABNT) NBR 6023, que deve ser consultada para outros tipos de documentos aqui não exemplificados.

Exemplos de Apresentação dos Autores nas Referências:
BONAGURA, J. D. (**um autor**)
SANTOS, J. A.; MELLO, M. R. (**dois autores**)
BENNETT, B. T.; ABEE, C. R.; HENRICKSON, R. (**três autores**)
VILELA, D.; MARTINS, C. E.; BRESSAN, M.; CARVALHO, L. A. [...] (**quatro autores ou mais**) ou VILELA, D. et al. (sem itálico).

Exemplo de periódico
2 KOTZEKIDOV, P.; BLOUKAS, J. G. Effect of protective cultures and packaging film permeability on shelflife of sliced vacuum-pocked cooked ham. **Meat Science**, v. 42, n. 3, p. 333-345, 1996.

Exemplo de livro
2 HALLIWELL, R. E. W.; GORMAN, N. T. **Veterinary clinical immunology**. London: W. B. Saunders, 1989. 548 p.

Exemplo de autor diferente para o livro e capítulo
3 FENNER, W. R. Avaliação neurológica dos pacientes. In: ETTINGER, S. J. **Tratado de medicina interna veterinária**. 3. ed. São Paulo: Manole, 1992. p. 577-606.

Exemplo de mesmo autor para o livro e capítulo
4 THORTON, H. Deleterius changes in meat. In: THORTON, H. **Aspects of meat inspection**. London: Thindall & Cassel, 1973. p. 63-72.

Exemplo de tese
5 BIRGEL, E. H. **Estudo do quadro eritrocitário de caprinos (*Capra hircus*, L.) normais criados no Estado de São Paulo**: influências de fatores raciais, sexuais, etários e alimentares. 1973. 92 f. Tese (Livre Docência) - Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 1973.

Exemplo de evento
6 OLIVEIRA, C. A. Hormonoterapia em cadelas e gatas. In: CONGRESSO BRASILEIRO DE REPRODUÇÃO ANIMAL, 9., 1991, Belo Horizonte. **Anais...** Belo Horizonte: Colégio Brasileiro de Reprodução Animal, 1991. p. 100-111.

Exemplo de livro eletrônico
7 POORE, M. H. **Alternative feeds for beef cattle**. North Carolina: North Carolina Corporative Extension Service, 1994. Disponível em: <http:// www.ces.ncsu.edu/drought/dro-28.html>. Acesso em: 23 abr. 2007.

Exemplos de artigos de periódicos eletrônicos
8 MENDONÇA JR., C. X.; MARTINS, A. P.; MORI, A. V.; SILVA, A. B.; MORI, C. S. Efeito da adição de óleo de peixe à dieta sobre o desempenho e níveis de lípides plasmáticos e de colesterol no ovo de galinhas poedeiras. **Brazilian Journal of Veterinary Research and Animal Science**, v. 37, n. 1, 2000. Disponível em: <http://www.scielo.br/ cgi_bin/wxis.exe/iach/scielo>. Acesso em: 31 jan. 2001

6 - Citações: utilizar o Sistema Numérico. As citações devem ser feitas por numeração única e consecutiva em sobrescrito, utilizando-se algarismos arábicos, remetendo à lista de referências na mesma ordem em que aparecem no texto. Quando indispensável para a compreensão do texto, combinar o(s) sobrenome(s) do(s) autor(es) com a indicação do número. Neste caso, a citação será pelo sobrenome de cada autor ou pelo nome da entidade responsável que aparece na respectiva referência. Quando se tratar de três autores, todos devem ser citados. No caso de mais de três autores, a citação deve ser acompanhada pelo sobrenome do primeiro autor seguido da expressão et al. (sem itálico). Se a citação estiver inserida no texto utilizar letras maiúsculas e minúsculas; se estiver entre parênteses utilizar somente letras maiúsculas. Exemplos:

Um autor
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Dois autores
Soares e Alves[13] ou (SOARES; ALVES[13])

Três autores
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INSTRUCTIONS TO AUTHORS

Mission and Editorial policy

The *Brazilian Journal of Veterinary Research and Animal Science* publishes scientific complete articles and high quality previous notes relative to the field of veterinary medicine as well as to veterinarians. Studies of the basic and applied sciences relative to veterinary medicine are welcome. Only previous notes that address matters of veterinary science and provide supporting scientific evidences will be published. The manuscripts will be analyzed by the Editorial Board, provided they adhere to the manuscript guidelines.

It is the official publication of Fundação Medicina Veterinária – FUMVET. The journal follows the guidelines established by the Editorial Board designated by the Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo. Articles and their concepts are sole responsibility of author (s).

Articles will be accepted for publication provided they adhere to the Author's guidelines and receive approval from the Scientific Committee and the Editorial Board, after hearing the reviewers.

Reviewers maintain confidentiality of the information presented in the manuscript. Reviewers should render their opinions in an unbiased and ethical manner. Identity of reviewers is kept secret.

Once an article is approved for publication by the Scientific Committee, a fee (R$) will be charged which will be calculated on the basis of number of pages. All authors should sign a form declaring they are aware and obliged to this payment when submitting a manuscript. After approval for publication, provided no payment was made, manuscript course of action will stop and manuscript returned to the authors.

Historical background

This periodical was founded in 1938 by the Faculdade de Medicina Veterinária which was part of Universidade de São Paulo. The periodical's former name was Revista da Faculdade de Medicina Veterinária da Universidade de São Paulo. In 1974, its name was changed to Revista da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, following the name change of the school. In 1990, its name was again changed, bearing the present denomination of *Brazilian Journal of Veterinary Research and Animal Science*. In 2000, the journal became the official publication of the Fundação Medicina Veterinária – FUMVET, setting away the institutional link with the Faculdade de Medicina Veterinária e Zootecnia da USP.

Correspondence address

Manuscripts and all letters should be sent to the following address:
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Seção de Publicação
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Author's guidelines

Complete article

1 – Articles must describe original research and should be submitted only to Brazilian Journal of Veterinary Research and Animal Science. It is necessary to include a form, signed by all authors, in which authors agree to the payment of publication fees.

2 – Manuscripts should be limited to ten written pages following the structure depicted in the item #5. Page tables and figures are not counted.

3 – Articles must be written in Portuguese or in English.

4- Use only official nomenclature. The use of author-defined abbreviations and acronyms is discouraged. Abbreviations in the titles should be avoided.

5 – Articles must be structured within the following sections:

a) Introduction
b) Materials and Methods
c) Results
d) Discussion
e) Conclusions
f) References
g) Abstract/Key-words

The items Results, Discussion and Conclusions may be included in a single section, except otherwise indicated by the Editorial Board.

6 – Authors must submit two abstracts, one in Portuguese and another in English, which should consist of no more than 250 words. The Abstract is followed by up to five key-words which should correspond to words or expressions that identify the contents of the article.

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2 – Should be up to three typed pages.

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4- Use only official nomenclature. The use of author-defined abbreviations and acronyms is discouraged.

5 – Previous Notes must not be divided into major headings (Introduction, Materials and Methods, etc.), but should present two abstracts followed by key-words as it was described for complete manuscript submission, including a list of References.

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Unsolicited review manuscripts will not be accepted; however, a draft may be submitted to the Editorial Board, without previous consent, aiming at appropriateness for publication. The Editorial Board may invite renowned specialists to submit review manuscripts.
These reviews must adhere to the guidelines of a complete article, though without division into major headings (Introduction, Materials and Methods, etc.). They should present two abstracts followed by key-words as it was described for complete article submission, including a list of References.

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1 – Typesetting: manuscripts should be submitted in CD-ROM or 3½" high density diskette. They have to bear manuscript's title and author's name (s). Authors must submit three copies of the manuscript, including tables and references, double-space typed and pages numbered consecutively. Page size is A4 (21.0 ´ 29.7 cm) and 2.5 cm for all margins. Use only front page and fixed character width (Times New Roman 10 cpi). Illustrations and legends must come on separate sheets. Authors must choose Microsoft Word as text editor.

2 – Cover page: all manuscripts must include a cover page containing the manuscript's title, author's name (s) and institution. In the footnote should be given the complete address (including e-mail) of the corresponding author. Care should be observed that author's identification is supposed to appear only in the cover page to assure unbiased and ethical reviews. If the work received financial assistance from the industry, mention to it as well as acknowledgment should be provided.

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4 – Illustrations (photographs, graphics, drawings or artwork): illustrations must be numbered consecutively in Arabic numbers and be cited in the text as Figures. The photographs shall be identified only by the manuscript's title and on the back contain description of correct positioning in the text. Graphics, drawings or artwork should be provided in separate sheets, identified by the manuscript's title and respective legends. All illustrations must be provided in three copies. Be sure that each mean and some index of variation is presented in the figures, the figure legend, or the text. Drawings and other artworks must have good technical quality and artistically accepted (in the case they are computer generated, printed originals must be included). Upper limit of figures per work is nine and it is suggested the following distribution: three photographs, three graphics and three drawings or artworks. Beyond this limit expenses with reproduction shall be the author's responsibility. Color photographs, independently of number, will be charged.

5- References: they must appear in the order they were cited in the text. Periodical or journal names must be uniform, preferentially not abbreviated. In the case, periodical or journal names are abbreviated, follow the NBR 6032. For references that are not illustrated in this Instructions to Authors follow instructions contained in the NBR 6023/2002. Indicate authors by last name, in capital letters, followed by initials of first and middle names. Authors are separated by semicolon and space.

Examples: BONAGURA, J. D. [...] (1 author)
SANTOS, J. A.; MELLO, M. R. [...] (2 authors)
BENNETT, B. T.; ABEE, C. R.; HENRICKSON, R. [...] (3 authors)
VILELA, D.; MARTINS, C. E.; BRESSAN, M.; CARVALHO, L. A. [...] (4 authors or more) **or** VILELA, D. et al. [...].

Example of periodical or journal
KOTZEKIDOV, P.; BLOUKAS, J. G. Effect of protective cultures and packaging film permeatibility on shelf-life of sliced vacuum-pocked cooked ham. **Meat Science**, v.42, n.3, p. 333-45, 1996.

Example of book
HALLIWELL, R. E. W.; GORMAN, N. T. **Veterinary clinical immunology**. London: W. B. Saunders, 1989. 548 p.

Example of chapter and book with different authors
FENNER, W. R. Avaliação neurológica dos pacientes. In: ETTINGER, S.J. **Tratado de medicina interna veterinária. 3. ed. São Paulo: Manole, 1992**. p. 577-606.

Example of same authorship for chapter and book
THORTON, H. Deleterius changes in meat. In: THORTON, H. **Aspects of meat inspection**. London: Thindall & Cassel, 1973. p. 63-72.

Example of thesis
BIRGEL, E. H. **Estudo do quadro eritrocitário de caprinos (Capra hircus, L.) normais criados no Estado de São Paulo**: influências de fatores raciais, sexuais, etários e alimentares, 1973. 92 f. Tese (Livre Docência) - Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo.

Example of meeting
OLIVEIRA, C. A. Hormonoterapia em cadelas e gatas. In: CONGRESSO BRASILEIRO DE REPRODUÇÃO ANIMAL, 9., 1991, Belo Horizonte. **Anais...** Belo Horizonte: Colégio Brasileiro de Reprodução Animal, 1991. p. 100-111.

Example of electronic publication
AUTHOR. **Title**. Edition. Local of publication: Publisher, year of publication. (Type and characteristics of midia support, producer, citation date, availability and access).

ONLINE
POORE, M. H. **Alternative feeds for beef cattle**. North Carolina: North Carolina Corporative Extension Service, 1994. Disponível em: <http://www.ces.ncsu.edu/drought/dro-28.html>. Acesso em: 23 abr. 1997.
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Example of articles from electronic journals
AUTHOR. TITLE OF ARTICLE. **Title of periodical**, volume, issue (number), pages, year of publication. (Type and characteristics of midia support, producer, citation date, availability and access).

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MENDONÇA JR., C.X.; MARTINS, A.P.; MORI, A.V.; SILVA, A.B.; MORI, C.S. Efeito da adição de óleo de peixe à dieta sobre o desempenho e níveis de lípides plasmáticos e de colesterol no ovo de galinhas poedeiras. **Brazilian Journal of Veterinary Research and Animal Science**, v.37, n.1, 2000. Disponível em: <http://www.scielo.br/cgi_bin/wxis.exe/iach/scielo>. Acesso em: 31 jan. 2001
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Examples: According to Yanaguita[9] [...]
[...] (YANAGUITA[9]) [...]
Bennett, Abee and Henrickson[12] [...]

Vilela, Martins, Bressan and Carvalho[26] [...]
(4 authors or more) **or** (VILELA et al.[26]) [...]