academicJournals

Vol. 9(42), pp. 1002-1008, 15 November, 2015
DOI: 10.5897/AJPP2015.4408
Article Number: B4DCA3256131
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Gender-related differences in pharmacokinetic parameters of tramadol following intravenous and subcutaneous administration in dogs

Buhari Salisu[1]*, Kalthum Hashim[2], Goh Yong Meng[3] and Gan Siew Hua[4]

[1]Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Nigeria.
[2]Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, 16100 Kota Bharu Kelantan, Malaysia.
[3]Department of Veterinary Preclinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[4]Human Genome Centre, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian Kelantan, Malaysia.

Received 12 July, 2015; Accepted 21 October, 2015

The kinetics of tramadol at 3 mg/kg was studied in twenty four local dogs (twelve males and twelve females), after a single intravenous and subcutaneous dose administration. Three milliliters of blood from the jugular vein were collected before and at 2, 5, 10, 15, 30 and 45 min, 1, 1.5, 2, 2.5, 3, 4, 6, 8 and 9 h post administration of tramadol from both groups with the exception of 2 min for subcutaneous group. The collected blood samples were analyzed using a high performance liquid chromatography. In male dogs, the maximum plasma concentration (Cmax) was attained (Tmax) much faster (0.17 h) and systemic bioavailability was higher (29.65±11.7%) than in female dogs with 15.68±4.19%. On the other hand, AUC, $t_{1/2\alpha}$, $t_{1/2\beta}$ $Vd_{(ss)}$ were not significantly different between male and female dogs. These findings suggest the presence of some differences in the kinetics of tramadol between the male and female dogs.

Key words: Tramadol, pharmacokinetics, gender-difference, dogs.

INTRODUCTION

Gender is the most important factor in mammalian development and response to exogenous agents (Maris et al., 2010). The common surgical procedure is ovariohysterectomy and involves female dogs (Kongara et al., 2010) hence male dogs are underrepresented. Unequal representation of male dogs in frequent surgical and clinical trials has caused a relative paucity of data toevaluate possible gender differences in tramadol pharmacokinetics. Gender differences in the pharmacokinetics of some drugs are known to exist

*Corresponding author. E-mail: msbuhari7@gmail.com.

(Franconi et al., 2007; Soldin and Mattison, 2009). Evaluation of these gender differences is of clinical importance in designing effective post-surgical p pain management protocols. In dogs, unlike in rats (Liu et al., 2003) and humans (Ardakani and Rouini, 2007), gender dependency of pharmacokinetics of tramadol has not been investigated in detail. Recent clinical and *in vitro* evidence related the higher expression of cytochrome P450 (CYP) 3A4 in females (Lamba et al., 2010; Yang et al., 2010) to gender related differences. Furthermore, gender related difference in systemic exposure and related pharmacokinetic parameters of tramadol enantiomers were reported among Chinese volunteers (Hui-chen et al., 2004). The high variability in the tramadol pharmacokinetic properties was partly related to CYP2D6 and MDR1 polymorphism. Variation within the extensive metabolizer phenotype based on the number of functional alleles was observed. CYP2D6 activities were reported to be higher in males than in females, leading to higher Cmax and AUC of the metabolites (M1) (Ardakani and Rouini, 2007). Contrary to that, Hui-chen et al. (2004) found higher values in females than in male volunteers. The main objective of this study was to determine whether pharmacokinetic parameters of tramadol differed in male and female dogs.

MATERIALS AND METHODS

Twenty four local dogs of both sexes; twelve males weighing between 15 and 22 kg (average 18.5±2.2 kg), and twelve females of between 12.5 and 18 kg (average 15.75±1.9 kg), aged between 1.5 and 4 years (mean 2.92±0.82 years), and aged between 1.5 and 3 years (mean 2.33±0.6) years were used for the study. They were obtained and kept separately, and were certified healthy based on physical and clinical examination prior to the study. On the morning of study, a 20 gauge $1_{1/4}$ inch sterile catheter (Terumo, Somerset NJ, USA) was placed and secured in the cephalic vein. A baseline 2 ml venous blood sample was collected from each dog before tramadol administration. The dogs were randomly divided into four groups of equal number and were fasted for 12 h prior to beginning of the study but had access to water until two hours to tramadol administration. Group I and II are male dogs and received 3 mg/kg tramadol intravenously and subcutaneously respectively while III and IV are female dogs also received 3 mg/kg tramadol intravenously and subcutaneously respectively. The experimental procedures were approved by the Universiti Putra Malaysia Animal Care and Utility Committee (UPM/FPV/PS/3.2.1.551/AUP-R86).

Sample collection for pharmacokinetic analysis

An 18 gauge jugular catheter was placed in each dog approximately 2 h prior to each study period. Three ml blood were taken before and at 2, 5, 10, 15, 30 and 45 min, 1, 1.5, 2, 2.5, 3, 4, 6, 8 and 9 h post administration of tramadol from groups with exception 2 min among the subcutaneous groups. Prior to removing blood samples for pharmacokinetic analysis, 1 ml sample of blood was removed from the jugular catheter and discarded. Catheters were flushed with 1 ml of sterile saline following each sample collection. Samples were placed in a plane tube (Becton Dickinson, Franklin Lanes, New Jersey, USA) and allowed to stand at room temperature for 30 min to clot before immediately placed on ice. Samples were carefully and accurately labeled using a permanent marker. The blood samples were centrifuged at 1000 × g for 10 min, and the separated serum samples were kept frozen at -80°C until analysis within 6 months.

Serum tramadol extraction using solid phase extraction (SPE) method

Serum extraction was accomplished with disposable non-end-capped solid phase extraction cartridges. The columns were conditioned with 1 ml methanol, followed by 1 ml of diionised distilled water (DDH_2O). One milliliter of serum sample was loaded into each column. The columns were washed three times with 1 ml of DDH_2O, followed by washing with 250 µl of acetonitrile (ACN):ethylacetate (EtAc) (60:40) combination for three times. Columns were eluted four times with 250 µl of the mixture of ACN: EtAc (60:40) with added Triethylamine 1%. The eluent were collected into 10 ml falcon tubes and a volume of 50 µl of phenacetin was spiked from a 20 µg/ml working solution as an internal standard. The mixture was vortexed and dried under nitrogen stream heated to 40°C to facilitate evaporation. The sample was reconstituted in 70 µl of mobile phase and then filtered through a 4 mm nylon syringe filter, 0.4 µm before being injected into the high performance liquid chromatography (HPLC) system.

Serum tramadol assay using HPLC

Serum tramadol was analyzed by reverse phase HPLC with ultraviolet (UV) detection. High performance liquid chromatography is flexible and sensitive and can detect the tramadol at lower doses and from very small samples, which is really useful in clinical trials (Gan et al., 2002). Detection limit with HPLC was found to be as low as 20 ng/ml (Kukanich and Papich, 2004). The repeatability of the method for tramadol estimation reflects its precision in determining tramadol biotransformation (Gan et al., 2002). In a study on normal dogs Kongara et al. (2009) demonstrated the repeatability of HPLC for tramadol assay. Also, a large number of trials that used HPLC for quantitation of tramadol demonstrated the reliability of the method (Kukanich and Papich, 2004; Kubota et al., 2008). The HPLC system consisted of a quaternary pump, degasser, automated sampler, and UV detector which was set at 218 nm. The analytical column was Agilent Zorbax reverse phase, with a particle size of 5 µm and diameter and length of 4.6 x 250 mm. The control of the HPLC system and data collection was achieved by use of an IBM-compatible computer equipped with Agilent LC ChemStation software. The HPLC method was based on previously published study (Gan and Ismail, 2001). To achieve separation, the Agilent Zorbax RP-C18 column was heated to 40°C. The mobile phase was a mixture of 70% phosphate buffer (0.01 M), 30% acetonitrile with addition of 0.1% triethylamine (v/v), and adjusted to a pH of 5.9. The phosphate buffer was prepared fresh daily by dissolving 1.36 g of KH_2PO_4 (HPLC grade, Fisher Scientific Loughborough, Leicestershire LE11 5RG UK) into 1000 ml DD H_2O. The final mixture was filtered under vacuum through a 0.45 µm cellulose filter (Sartorius, Germany) and sonicated for 20 min. A flow-rate of 0.75 ml/min was chosen and an injection volume of 25 µl for each reconstituted sample throughout.

Standard curve for tramadol

Standard curves for tramadol were prepared daily. The pure

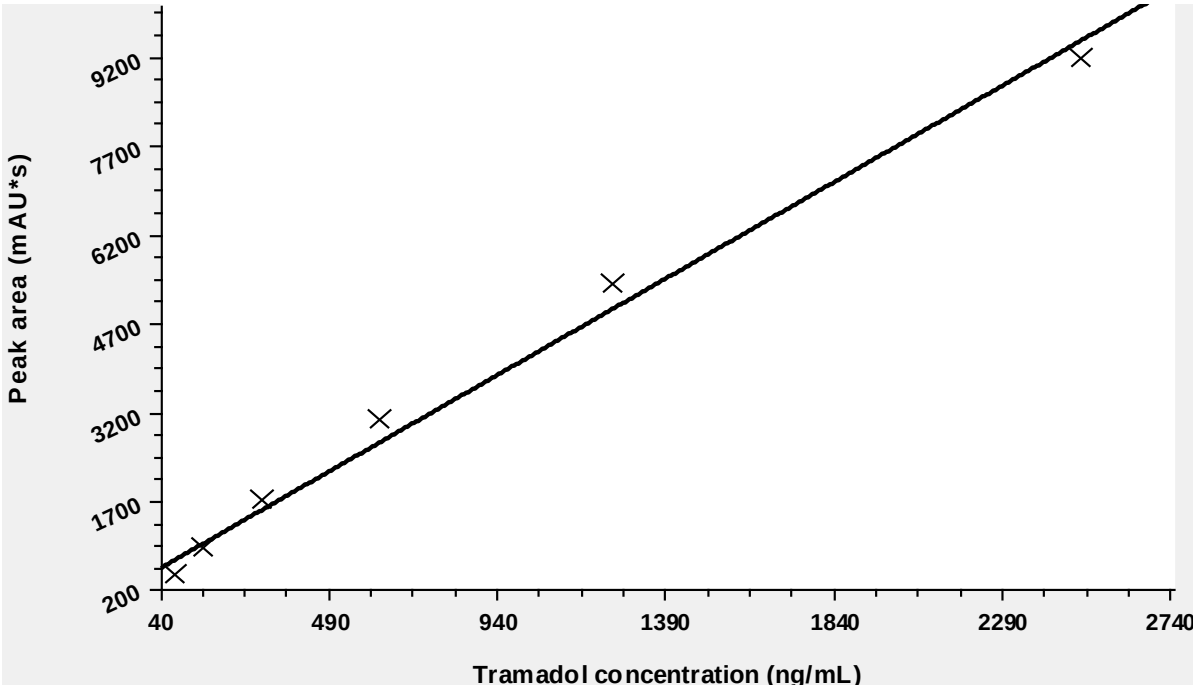

Figure 1. Graph of known concentrations of tramadol range from 40 to 2500 ng/ml (X-axis) plotted against peak area (Y-axis) determined (r = 0.99).

dry tramadol (Sigma-Adrich Co., 3050 Spruce Street, St. Louis, M063103 USA) was diluted in mobile phase to produce a concentration range from 40 to 2500 ng/ml and directly injected into the HPLC system for analysis. Daily calibration with a coefficient of determination (r^2) value greater than 0.99 was accepted.

Preparation of daily quality control samples

Quality control samples were prepared and run daily before injecting experimental sample. Canine frozen serum samples were left on the bench to thaw naturally and were vortexed prior to use. The curves were prepared by fortifying pooled canine serum with pure dry tramadol to produce a concentration range from 40 to 2500 ng/ml, while phenacetin was added at 20 µg/ml throughout. Preparation and processing of the fortified calibration samples were exactly as described for the incurred serum samples.

Pharmacokinetic analysis

Pharmacokinetic variables of tramadol following administration were calculated using a pharmacokinetic computer software program (WinNonlin 6.2.1. Pharsight Corp., Mointain View CA, USA). A weight factor of ($1/y^2$) was applied to the pharmacokinetic calculations. The best fit model for compartmental analysis was determined by residual plots and Aikake's information criterion. An open two-compartment model with central compartment best described the decline in tramadol plasma concentration following intravenous administration, and a standard non-compartmented model following subcutaneous administration. Values for total body clearance (Cl), volume of distribution (Vd), area under the plasma concentration curve (AUC), plasma distribution half-life a ($aT_{1/2}$), plasma clearance half-life b($b\ T_{1/2}$), intercept of the distribution phase (A), intercept of the elimination phase (B), rate constant associated with distribution (alpha), and rate constant associated with elimination (beta) were derived.

Statistical analysis

Statistical analysis was performed using the SPSS program (IBM® SPSS software Inc. version 16, New York, USA). The results are expressed as the means ± SD. The pharmacokinetic parameters were compared using an independent *t*-test between the groups. A *p*-value of less than 0.05 was considered to be statistically significant.

RESULTS

The linear concentration range for tramadol analysis was 40 to > 2500 ng/ml (39.625, 78.125, 156.25, 312.5, 625, 1250, 2500) ng/ml, (n = 5) (r^2 > 0.99). The limit of detection and quantitation were found to be 10 and 50 ng/ml respectively (Figure 1). Mean retention time for phenacetin (internal standard) was 6.98 min (Figure 2) and tramadol was 5.09 min (Figure 3). Dog experimental tramadol plasma chromatogram 5 min and 6 h after administration are presented in Figures 4 and 5 respectively.

No adverse effects were observed after administration of tramadol HCl at 3 mg/kg. All dogs appeared mildly sedated after administration and a dog showed sign of nausea (salivating) but stopped after about 5 min. A female dog became very aggressive and was replaced with another female dog.

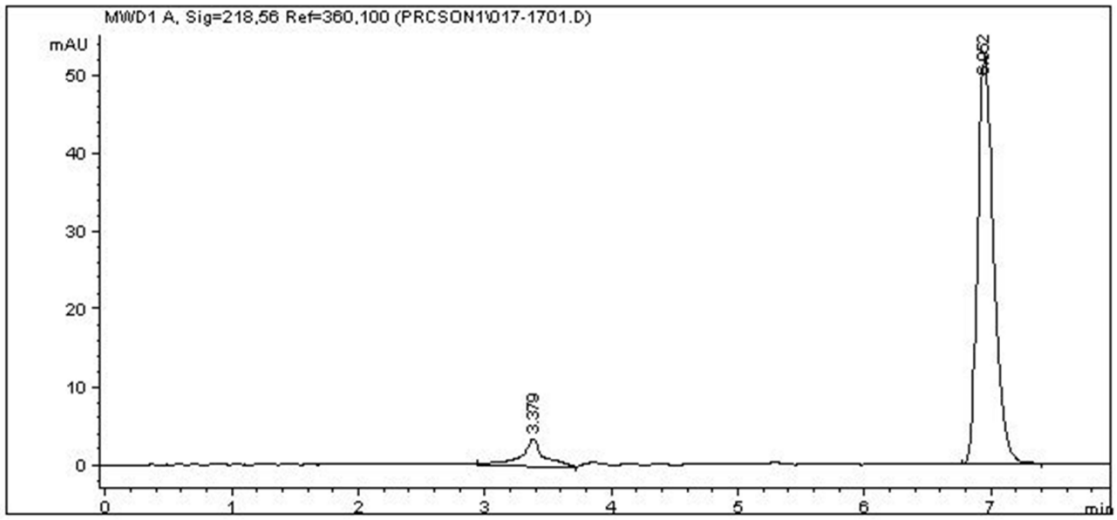

Figure 2. Representative chromatogram obtained from phenacetin injected into HPLC at 20 µg/mL as an internal standard.

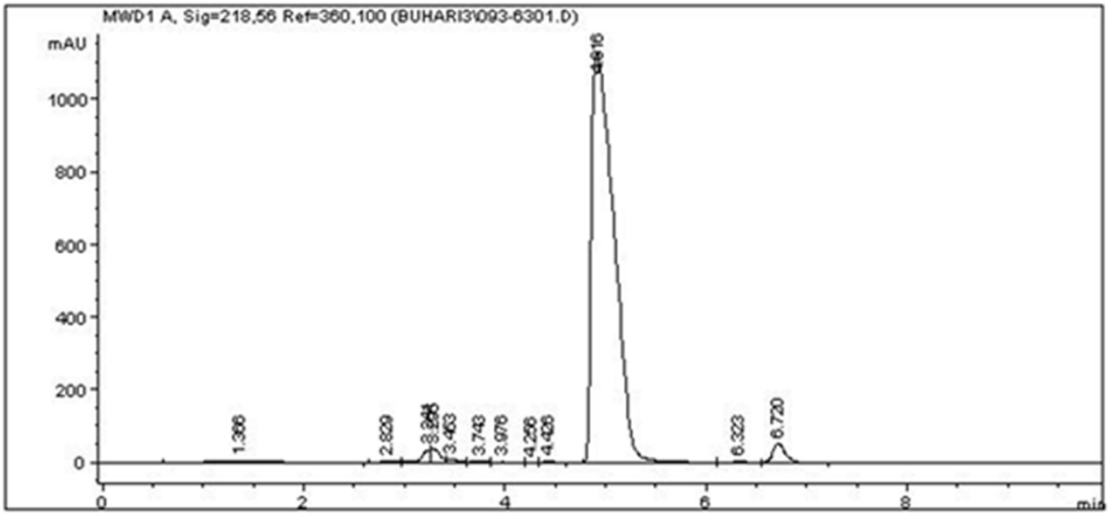

Figure 3. Representative chromatogram obtained from tramadol and phenacetin injected into HPLC at 5000 and 20 µg/ml, respectively.

Influence of gender on pharmacokinetic of tramadol in dogs

Similar results were obtained after tramadol pharmacokinetic analysis for gender difference subgroups. However, it is interesting to note that systemic bioavailability was higher among the male dogs (29.65±11.7%) than female dogs with 15.68±4.19%. This resulted to a significantly higher rate of movement from compartment 1 to compartment 2 among the female dogs (13.34±12.58 l/h) than the male dogs (5.99±4.1 l/h). Maximum plasma concentration (Cmax) was attained (Tmax) much faster (0.17 h) among the male dogs compare to the female dogs (0.75 h). On the other hand, AUC, $t_{1/2\alpha}$, $t_{1/2\beta}$ $Vd_{(ss)}$ were not significantly different between male and female dogs. These findings suggest that the tramadol is influenced to a lesser extent by gender differences. The pharmacokinetic parameters are summarized in Table 1.

DISCUSSION

The gender related pharmacokinetic parameters derived

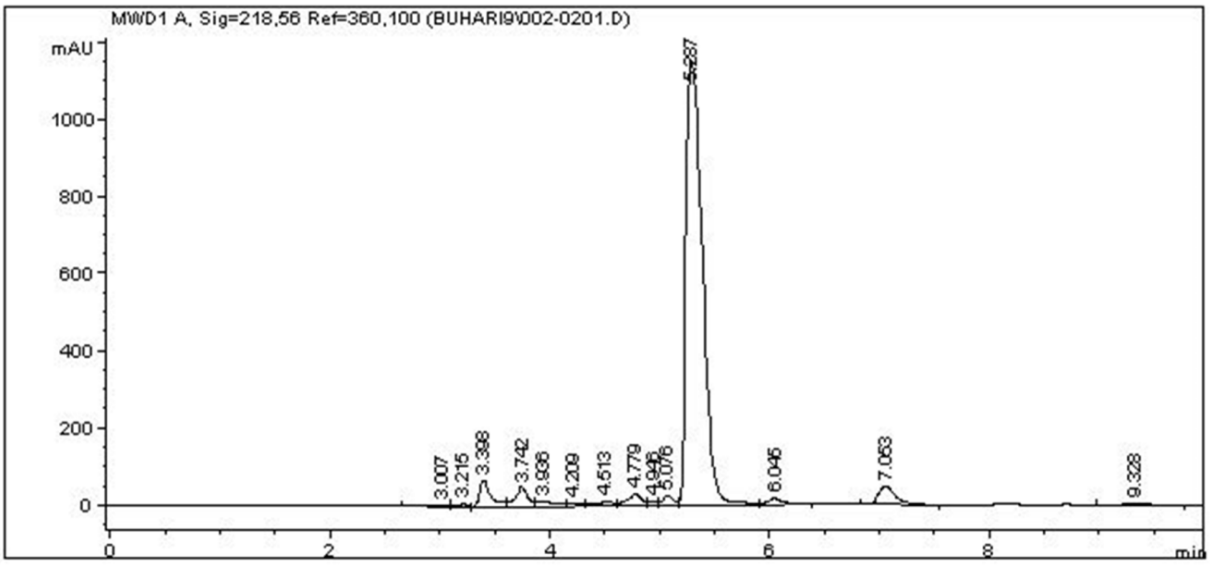

Figure 4. Tramadol plasma representative chromatogram obtained from dog 5 min following administration.

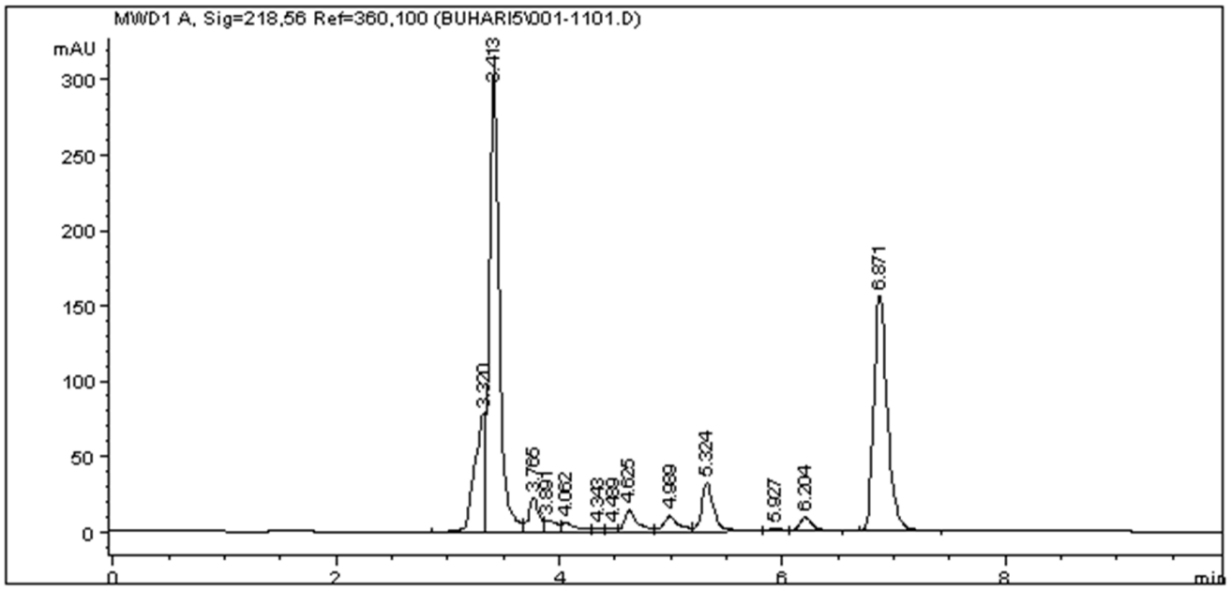

Figure 5. Tramadol plasma representative chromatogram obtained from dog 6 h following administration.

for tramadol in our study differ somewhat to those recently published by Cagnardi et al. (2011) in cats. Our results showed a significantly faster time to reach high plasma tramadol concentration (0.17 ± 0.01 h) in male dogs compare with relatively slower (0.75±0.01 h) among the female dogs. Rate of movement of tramadol from first compartment to the second compartment was significantly lower (5.99±4.1) in the male dogs compare with 13.34±12.58 found among the female dogs, and subsequently higher systemic bioavailability (29.65±11.7%) among the male dogs versus lower values (15.68±4.14%) observed in the female dogs. However, a wide inter-individual variation was observed among the female dogs (range from 11.3 to 24.4%). Unlike Cagnardi et al. (2011) who reported no sex-related differences in tramadol pharmacokinetics in cats and Ardakani and Rouini (2007) among human volunteers. Hui-chen et al. (2003) observed a significant gender variation in pharmacokinetics of trans-tramadol in rats. However, they observed systemic exposure among the female rats compared with the male rats. In another study by Djurendic-Brenesel et al. (2010), significantly higher

Table 1. Pharmacokinetic parameters of tramadol (Mean ± SD) following intravenous (3 mg/kg) and subcutaneous (3 mg/kg) administration in both male and female dogs.

Parameter	Group I (n = 6)	Group II (n = 6)	Group III (n = 6)	Group IV (n = 6)
A (ng/ml)	195.07±40.82[a]	NA	657.3±462.5[b]	NA
B (ng/ml)	173.13±23.93	NA	148.68±47.28	NA
α (l/h)	11.42±3.8[a]	NA	45.61±39.19[b]	NA
β (l/h)	1.13±0.29	NA	1.12±0.2	NA
λz (l/h)	1.23±0.44	0.65±0.16[a]	1.43±0.21	1.09±0.37[b]
$t_{1/2}$λz (h)	0.58±0.16	1.13±0.34[a]	0.49±0.07	0.69±0.21[b]
$t_{1/2\alpha}$ (h)	0.07±0.03	NA	0.04±0.04	NA
$t_{1/2\beta}$ (h)	0.65±0.16	NA	0.64±0.11	NA
C_0 (ng/ml)	294.17±22.68[a]	NA	333±120.48[b]	NA
Cmax (ng/ml)	245.57±26.02[a]	NA	127.16±18.69[b]	NA
Tmax (h)	0.17±0.01[a]	NA	0.75±0.01[b]	NA
MRT (h)	0.85±0.22[a]	1.52±0.37[b]	0.76±0.18[a]	1.39±0.19[b]
Cl_T (mL/min/kg)	17.71±5.02	17.17±4.32	21.06±9.34	16.53±5.29
$Vd_{(ss)}$(L/kg)	14.18±1.56	NA	14.37±4.96	NA
$AUC_{0-\infty}$ (h*ng)/ml	179.52±44.47	177.19±63.28	177.61±85.16	196.01±57.66
$AUMC_{0-\infty}$ (h*h*ng)/ml	159.34±75.85[a]	276.21±81.44[b]	140.01±75.25[a]	281.05±11.75[b]
K10 (l/h)	2.54±1.07	NA	4.33±2.1	NA
K12 (l/h)	5.99±4.1[a]	NA	13.34±12.5[b]	NA
K21 (l/h)	7.61±2.97	NA	6.12±1.46	NA
K10 $t_{1/2}$ (h)	0.34±0.12	NA	0.17±0.1	NA
V1 (L/kg)	7.7±1.62	NA	3.3±2.93	NA
V2 (L/kg)	5.91±1.74	NA	8.92±2.81	NA
F (L/kg)	NA	29.65±11.7[a]	NA	15.68±4.19[b]

[a,b]means with different superscripts within rows different significantly at p=0.05.

Abbreviations: Group I = male dogs treated with a single dose of 3 mg/kg tramadol intravenously; Group II = male dogs treated with a single dose of 3 mg/kg tramadol subcutaneously; Group III = female dogs treated with a single dose of 3 mg/kg tramadol intravenously; Group IV = female dogs treated with a single dose of 3 mg/kg tramadol subcutaneously; NA not applicable; λz = first-order rate constant; t½ λz = half-life of the terminal portion of the curve; MRT = mean residence time; Cl_T = total body clearance; Vdss = volume of distribution at steady state; AUC0-∞ = area under the curve from 0 to infinity; AUMC0-∞ = area under the first moment curve from 0 to infinity; CO = concentration at time 0; Cmax = maximum concentration; tmax = time to maximum concentration; t½α = distribution half-life; t½β = elimination half-life; α = rate constant associated with distribution; β = rate constant associated with elimination; A = intercept for the distribution phase; B = intercept for the elimination phase; K10 = elimination rate from compartment 1; K12 = rate of movement from compartment 1 to compartment 2; K21= rate of movement from compartment 2 to compartment 1; K10 $t_{1/2}$ = half-life of the elimination phase; V1= volume of compartment 1; V2 = volume of compartment 2; F = bioavailability.

plasma opiates were measured among male rats over the female rats. This finding is in concordance with our observation, suggesting a faster passage of the drug from blood to the organs in female dogs. Gender relatedvariability in the pharmacokinetic properties of tramadol has been partly related to CYP2D6 and MDR1 polymorphism in humans. In addition, variation within population of extensive metabolizers (EM) phenotype was observed based on number of functional CYP2D6 alleles (Ardakani and Rouini, 2007). The activity of CYP2D6 has been reported to be higher in males than in females (Tanaka, 1999). Contrary to this, Hui-chen et al. (2004) reported a higher rate of O-demethylation of tramadol mediated by CYP2D6 resulting in higher Cmax and AUC in females over the males volunteers.

Both distribution and elimination half-lives remain unaffected, and volumes of distribution and elimination rates were not significantly different between the gender groups. This is similar to the observation made by Cagnardi et al. (2010) in cats, and in accordance with Ardakani and Rouini (2007) among human volunteers.

Conclusion

In conclusion, the clinical implications of these findings seem clear. If the aim is to achieve similar analgesic plasma levels of tramadol among male and female dogs it is unwise to give equal dose; not only that it takes faster time to reach high plasma tramadol concentration in male dogs but higher systemic bioavailability among the male dogs is achieved. If analgesia is to be achieved with tramadol administration, adjustment in the dose rate would have to be made to compensate for the poor bioavailability of the drug in female dogs. Overall, we conclude that there were some differences in the kinetics

between the male and female dogs.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Ardakani YH, Rouini MR (2007). Pharmacokinetics of tramadol and its three main metabolites in healthy male and female volunteers. Biopharma Drug Disp. 28(9):527-534.

Cagnardi P, Villa R, Zonca A, Gallo M, Beccaglia M, Luvoni GC, Vettorato E, Carlis S, Fonda D, Ravasio G (2011). Pharmacokinetics, intraoperative effect and postoperative analgesia of tramadol in cats. Res. Vet. Sci. 90(3):503-509.

Djurendic-Brenesel M, Mimica-Dukic N, Pilija V, Tasic M (2010). Gender- related differences in the pharmacokinetics of opiates. Forensic Sci. Int. 794(1-3):28-33.

Franconi F, Brunelleschi S, Steardo L, Cuomo V (2007). Gender differences in drug responses. Pharmacol. Res. 55(2):81-95.

Gan SH, Ismail R (2001). Validation of a high-performance liquid chromatography method for tramadol and o-desmethyltramadol in human plasma using solid-phase extraction. J. Chromatogr. B Biomed. Sci. Appl. 759(2):325-335.

Gan SH, Ismail R, Wan Adnan WA, Wan Z (2002). Method development and validation of a high-performance liquid chromatographic method for tramadol in human plasma using liquid- liquid extraction. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 772(1):123-129.

Hui-Chen L, Shu-Min J, Yong-Li W (2003). Gender-related differences in pharmacokinetics of enantiomers of trans-tramadol and its active metabolite, trans-O-demethyltramadol, in rats. Acta Pharmacol. Sin. 24(12):1265-1269.

Hui-chen L, Yang Y, Na W, Ming D, Jian-fang L, Hong-yuan X (2004). Pharmacokinetics of the enantiomers of trans-tramadol and its active metabolite, trans-O-demethyltramadol, in healthy male and female Chinese volunteers. Chirality 16(2):112-118.

Kongara K, Chambers JP, Johnson CB (2010). Electroencephalographic responses of tramadol, parecoxib and morphine to acute noxious electrical stimulation in anaesthetised dogs. Res. Vet. Sci. 88(1):127-133.

Kongara K, Chambers P, Johnson CB (2009). Glomerular filtration rate after tramadol, parecoxib and pindolol following anaesthesia and analgesia in comparison with morphine in dogs. Vet. Anaesth. Analg. 36(1):86-94.

Kubota R, Komiyama T, Miwa Y, Ide T, Toyoda H, Asanuma F, Yamada Y (2008). Pharmacokinetics and postoperative analgesia of epidural tramadol: A prospective, pilot study. Cur. Ther. Res. Clin. Exp. 69(1):49-55.

KuKanich B, Papich MG (2004). Pharmacokinetics of tramadol and the metabolite O-desmethyltramadol in dogs. J. Vet. Pharmacol. Ther. 27(4):239-246.

Lamba V, Panetta JC, Strom S, Schuetz EG (2010). Genetic Predictors of Interindividual Variability in Hepatic CYP3A4 Expression. J. Pharmacol. Exp. Ther. 332(3):1088-1099.

Maris AF, Franco JL, Mitozo PA, Paviani G, Borowski C, Trevisan R, Uliano-Silva M, Farina M, Dafre AL (2010). Gender effects of acute malathion or zinc exposure on the antioxidant response of rat hippocampus and cerebral cortex. Basic Clin. Pharmacol. Toxicol. 707(6):965-970.

Soldin OP, Mattison DR (2009). Sex Differences in Pharmacokinetics and Pharmacodynamics. Clin .Pharmacokinet 48(3):143-157.

Tanaka E (1999). Gender-related differences in pharmacokinetics and their clinical significance. J. Clin. Pharm. Ther. 24(5):339- 346.

Yang X, Zhang B, Molony C, Chudin E, Hao K, Zhu J, Gaedigk A, Suver C, Zhong H, Leeder JS, Guengerich FP, Strom SC, Schuetz E, Rushmore TH, Ulrich RG, Slatter JG, Schadt EE, Kasarskis A, Lum PY (2010). Systematic genetic and genomic analysis of cytochrome P450 enzyme activities in human liver. Genome Res. 20(8):1020-1036.