Effect of oral co-administration of frozen-dried grapefruit juice on pharmacokinetics of tramadol in dogs

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Summary

Tramadol is a centrally acting analgesic drug extensively metabolized in animal species. Its clinical response is mainly due to the M1 metabolite, poorly produced in dogs. Grapefruit-juice can inhibit the metabolism of different drugs in animals and humans. The aim of the present study was to evaluate the pharmacokinetics of tramadol and its major metabolites after co-administration of tramadol and frozen-dried grapefruit-juice. A balanced cross-over study was used involving six male Beagle dogs. They were administered with tramadol alone (5 mg/kg) or with tramadol (5 mg/kg) plus frozen-dried grapefruit-juice (10 g). The plasma concentration *vs* time curves showed significant differences during the first 4 h following drug administration. Tmax was at 1.33 and 1.70 h following tramadol and tramadol plus frozen-dried grapefruit-juice treatment, respectively. Significant differences were also shown in Cmax (490 *vs* 270 ng/ml) and AUC (11,610 *vs* 5,890 h·h·ng/ml). Significant differences between the treatments were shown in all the M1 parameters reported. M2 and M5 did not show significant differences after both administrations. In conclusion, the frozen-dried grapefruit-juice was shown to affect the plasma concentrations of M1, despite them being well below those reported in humans.

Key words: Tramadol, Metabolites, Frozen-dried grapefruit-juice, Pharmacokinetics, Dog

Introduction

Tramadol is a centrally acting analgesic drug that has been used clinically for the last two decades in humans. Tramadol is well tolerated with a low incidence of adverse side-effects in humans (Raffa *et al*., 1992). Tramadol has a dual mechanism of action: 1) it displays a low affinity for the mu- and delta-opioid receptors, and weaker affinity for the kappa-subtype, and 2) it also interferes with the neuronal release and re-uptake of serotonin and noradrenaline in descending inhibitory pathways (Raffa *et al*., 1992). The metabolism of this drug has been investigated in different species as rodents (Lintz *et al*., 1981), goats (de Sousa *et al*., 2008), cats (Pypendop and Ilkiw, 2008), dogs (KuKanich and Papich, 2004; Giorgi *et al*., 2009a, b, d), donkeys (Giorgi *et al*., 2009c), horses (Giorgi *et al*., 2007; Giorgi *et al*., 2010a) and alpacas (Giorgi *et al*., 2010b); similar metabolites are produced but in different amounts. The clinical response of tramadol is correlated to its metabolism, because of the different analgesic activities of its metabolites. O-desmethyl-tramadol hydrochloride (M1), the major active metabolite, has 200 times the affinity of tramadol for mu opioid receptors in humans (Raffa *et al*., 1992). The primary metabolites of Phase I, namely M1 and N-desmethyl-tramadol (M2), may be further metabolised to another metabolite, namely N-O-

didesmethyl-tramadol (M5) (Fig. 1). The lack of side effects, characteristic of opioid derivatives, and the absence of typical non-steroidal anti-inflammatory drugs side effects, suggest that tramadol is a potential molecule for long-term therapeutic use for chronic pain in animals. Despite its long-term use, the understanding and prediction of the time course of its pharmacological effects are still hampered by the presence of active metabolites and the co-existence of opioid and non-opioid mechanisms. Recently, tramadol was reported to be metabolised faster to inactive metabolites in goats (de Sousa *et al*., 2008), dogs (KuKanich and Papich, 2004; Giorgi *et al*., 2009a, b, d) donkeys (Giorgi *et al*., 2009c) horses (Giorgi *et al*., 2007; Giorgi *et al*., 2010a) and alpacas (Giorgi *et al*., 2010b) than in cats (Pypendop and Ilkiw, 2008) and dromedary camels (Elghazali *et al*., 2008). The clinical effectiveness of tramadol has been questioned in species that mainly metabolise this molecule to inactive metabolites, suggesting that this drug would not be suitable as an effective and safe treatment for pain in animals as it is in humans (Giorgi *et al*., 2007; 2009a, b, d; de Sousa *et al*., 2008; Giorgi, 2008). Pharmaceutical formulation seems to be related to the bioavailability of tramadol, since by the oral administration, tramadol undergoes through gastrointestinal first pass effect; immediate release tablets and sustained release capsules show bioavailability of 65% (KuKanich and Papich, 2004) and 10% (Giorgi *et al*., 2009d), respectively.

Co-administration of grapefruit-juice (GFJ) and some drugs (e.g cyclosporine, praziquantel, …) represents a relatively safe tool for "boosting" drug concentrations in dogs (Giorgi *et al*., 2003; Amatori *et al*., 2004; Hanley *et al*., 2010). Grapefruit juice (GFJ) has been demonstrated to inhibit the drug-metabolizing enzyme cytochrome P450 (CYP) 3A4, specifically located in the small intestine (Lown *et al*., 1997). Additional studies revealed the involvement of GFJ on the permeability of P-glycoprotein (P-gp) (Honda *et al*., 2004) and the organic anion transporting polypeptide activities (Kamath *et al*., 2005).

Various *in vitro* and *in vivo* investigations have implicated the furanocoumarins as the GFJ constituents responsible for CYP3A inhibition in humans (Paine *et al*., 2006). The components present in GFJ with the potential to interact with drugs still remain uncertain. The findings are

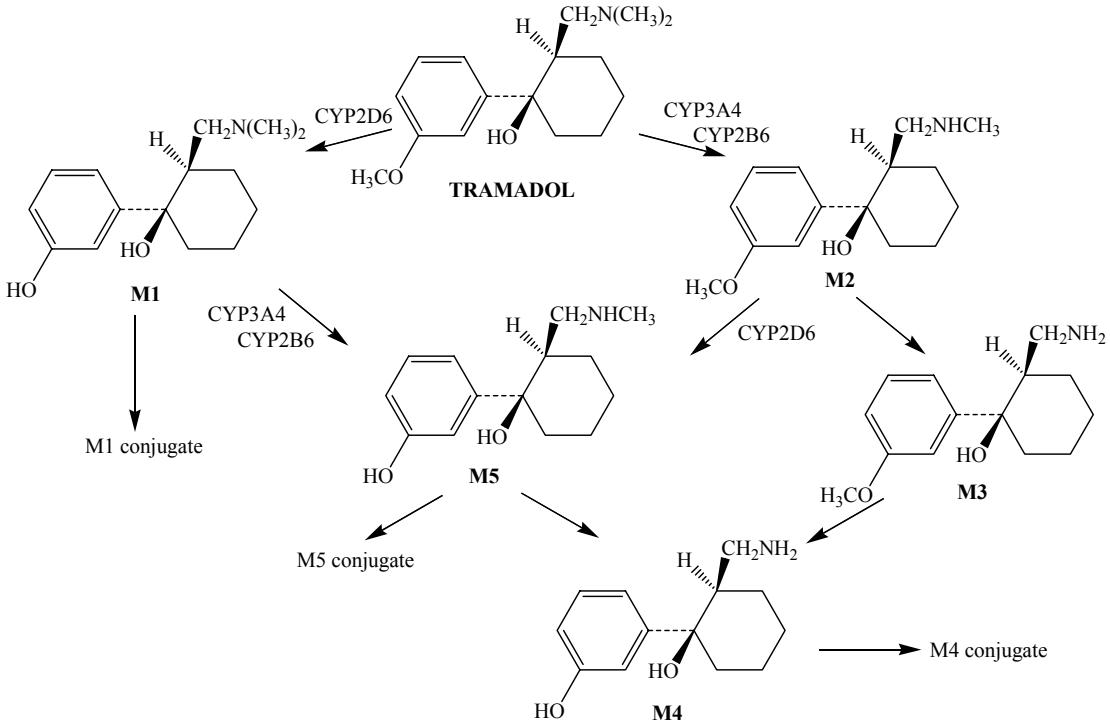


Fig. 1: Main metabolic pathways of tramadol (Lintz *et al*., 1981)

erratic: some results (Takanaga *et al*., 1998) suggest that GFJ and the components distinct from flavonoids (naringin and naringenin) significantly increase the bioavailability of certain drugs. Conversely, other studies found that 6′,7′-dihydroxybergamottin had a considerable effect reducing the secretion of the P-gp substrate (Wang *et al*., 2001).

While GFJ drug interactions have been extensively studied in humans, far fewer studies have been conducted in dogs (Giorgi *et al*., 2003; Amatori *et al*., 2004; Hanley *et al*., 2010). Hence, the aim of the present study was to test the co-administration of frozen-dried (FD) GFJ and tramadol in dogs, to evaluate if this therapy could enhance the plasma concentration of M1.

Materials and Methods

Tramadol hydrochloride and sotalol hydrochloride (internal standard [IS]) were obtained from Sigma-Aldrich (St. Louis, MO, USA). O-demethyltramadol hydrochloride (M1), N-demethyltramadol (M2), and O, N-didemethyltramadol (M5) were purchased from LGC Promochem (Milano, Italy). Acetonitrile, methanol, diethyl ether, dichloromethane, and 1-butanol (high-pressure liquid chromatography [HPLC] grade) were purchased from Merck (Darmstadt, Germany). Sodium dodecyl sulphate (SDS), sodium dihydrogen phosphate, and tetraethyl ammonium bromide (TEA) were analytical grade from BDH (Poole, UK). Deionised water was produced by a Milli-Q Millipore Water System (Millipore, MA, USA).

Frozen-dried grapefruit-juice preparation

Six fresh yellow grape-fruits were squeezed. The juice (about 800 ml) was quickly placed in ice and citric acid was added (1 g/100 ml GFJ) to prevent oxidation. Immediately 6 parts of GFJ (each of 100 ml) underwent the lyophilisation process lasting 24 h. The process was repeated twice to produce the required doses. Every 100 ml gave 10 g of residue (FD-GFJ) that constituted a single dose. The FD-GFJ was maintained under vacuum and at -20°C until the administration.

Animals and experimental design

Six male Beagle dogs, aged from 3- to 6-year-old and weighing from 18 to 23 kg, were previously determined to be clinically healthy on the basis of physical examination and full chemistry haematological analyses. The study protocol was approved by the ethics committee of the University of Pisa (Italy).

The test preparations were made according to an open balanced cross-over design: animals were randomly assigned to two treatment groups, using an open, single dose, two treatment, two period, randomized cross-over design. Each subject received a single oral dose of 5 mg/kg of tramadol in the morning after 12 h overnight fast, either alone (capsule prepared *ad subject*) or with FD-GFJ (10 g). The wash-out period was one week. A catheter was placed into the right cephalic vein to facilitate blood withdrawals.

Blood samples, 5 ml per sample, were collected at 0, 5, 15, 30, and 45 min and 1, 1.5, 2, 4, 6, 8, 10 and 24 h. The blood was then placed into collection tubes containing lithium heparin. The samples were centrifuged 3000 rpm within 30 min after collection and the harvested plasma was frozen at -20°C until detection of substances within 30 days.

Chromatography

Plasma tramadol, M1, M2 and M5 concentrations were evaluated by HPLC detection method according to Giorgi *et al*. (2007). Briefly, the HPLC system was an LC Workstation Prostar (Varian, Walnut Creek, CA, USA) consisting of an LC-10ADvp pump, CTO-10Avp column oven, SCL-10Avp system controller, and RF-10A spectrofluorometric detector. Data were processed by an LC solution Workstation (Varian Corporation). Chromatographic separation was performed on a Luna C18 ODS2 analytical column (150 mm × 2.1 mm inner diameter, 3-mm particle size) maintained at 25°C. The mobile phase consisted of acetonitrile:buffer (20 mM sodium dihydrogenphosphate, 30 mM SDS, and 15 mM TEA adjusted to pH = 3.9 with

phosphoric acid) (40:60, v/v) at a flow rate of 1.5 ml/min. Excitation and emission wavelengths were 275 and 300 nm, respectively. Validation data were previously reported (Giorgi *et al*., 2007).

Sample preparation

Samples were prepared by placing 1.0 ml plasma into a 15-ml polypropylene tube (Sarsedt, Verona, Italy) followed by 100 µl IS solution (8 µg/ml) and 0.5 ml 0.2 M borate buffer (pH = 9.3). After vortex-mixing, 7.0 ml extraction solvent (diethyl ether:dichloromethane:1-butanol 5:3:2) was added, then the tube was shaken for 20 min (100 osc/min) and centrifuged for 10 min at 3,400 rpm. Five mL of the organic layer was transferred to a clean 15-ml plastic conical tube, shaken with 200 µl back-extraction solvent (0.05 M H_2SO_4:acetonitrile 9:1) for 20 min (100 osc/min), and centrifuged for 10 min at 3,400 rpm. The aqueous phase (20 µl) was injected into the HPLC system.

Pharmacokinetic evaluation

The pharmacokinetic calculations were carried out with WinNonLin V. 5.1 program (Pharsight Corp., Cary, NC, USA). The $AUC_{0-\infty}$ was calculated with the log-linear trapezoidal rule. Cmax, the highest observed plasma concentration, and Tmax, the time required to reach Cmax, were obtained from the individual plasma concentration/time curves. Half-life (T1/2) was calculated from the slope of the logarithm of concentration versus time profile using at least the four terminal points of the curve. Changes in plasma concentrations of tramadol M1, M2 and M5 were evaluated by use of standard non-compartmental analysis and the relative pharmacokinetic parameters were determined with standard non-compartmental equations.

Statistical analysis

Normal distribution of data was assessed running a Shapiro-Wilk test. Statistical analyses were evaluated on the means of the different groups of treated animals using two independent samples t-test. The results were presented as mean (±SD). All the analyses were realized using GraphPad InStat (1998). In all the experiments differences were considered significant if the associated probability level was lower than 0.05 ($P<0.05$).

Results

Throughout the study no abnormality, definitely attributable to the drug tested, was observed, as assessed by the measurement of objective symptoms, vital signs, physical examination and hematological analyses.

Plasma profile *vs* time curves of tramadol, M1, M2 and M5 after both treatments are pictured in Figs. 2A, B, C and D. The animals have shown large variability in the plasma concentration of tramadol and metabolites. They were best fitted to a non-compartmental model.

The plasma observed concentration *vs* time curves of tramadol (Fig. 2A), were significantly different in both treatments for 4 h after administration. The Tmax values were reached about 1 and 2 h after the treatment of tramadol alone and co-administration of tramadol and FD-GFJ, respectively. In both treatments, at the 10th h the tramadol concentration values were very close to the LOQ (5 ng/ml) and at 24th h were under the LOD (0.5 ng/ml).

Larger differences were reported in M1 observed plasma concentrations (Fig. 2B). After co-administration of tramadol and FD-GFJ, the Tmax of M1 was reached within 30 min. Its concentration value result was significantly higher than the treatment with tramadol alone. During the elimination phase the plasma concentrations of M1 dropped down quickly and after 10 h were below the LOQ. In contrast, after the administration of tramadol alone, the Tmax of M1 was reached after 1 h. Its concentration value achieved the LOQ at 10th h. Anyway, these data could be unreliable due to both the low number of animals and the large variability among the subjects, and should be interpreted carefully.

Figures 2C and 2D depict the observed plasma concentration *vs* time curves for M2 and M5, respectively. In both administrations the curves seemed to match and no significant differences were reported in either treatment.

The predicted pharmacokinetic

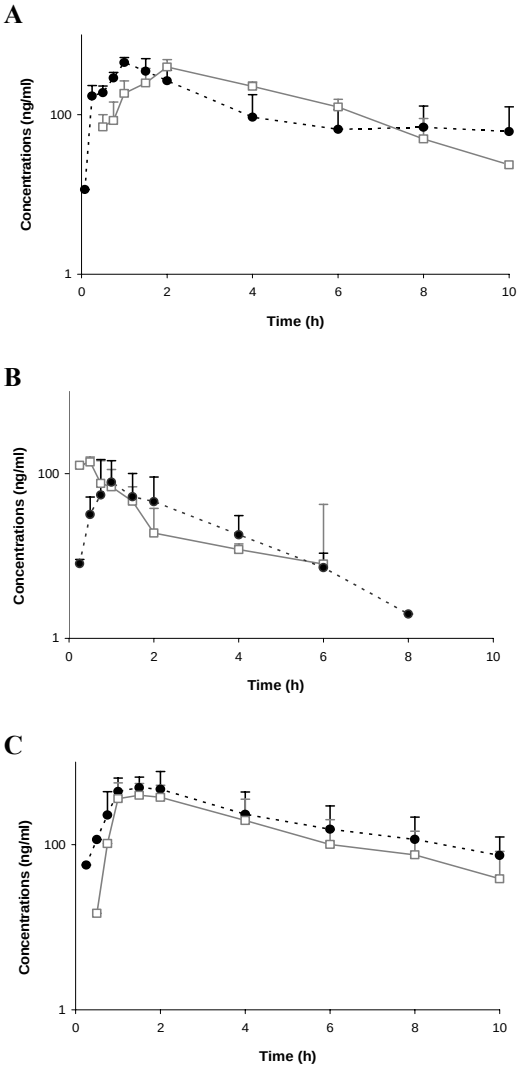
parameters of tramadol, M1, M2 and M5 are reported in Tables 1 and 2. Comparing the tramadol parameters after administration of tramadol and co-administration of tramadol and FD-GFJ, Tmax, showed a significant increase (1.33 ± 0.58 vs 1.17 ± 0.45 h, respectively), while Cmax and AUC showed a significant decrease (490 ± 260 vs 270 ± 210 ng/ml and 2,060 ± 1,630 vs 1,300 ± 460 h ng/ml, respectively).

Taking into account the predicted pharmacokinetic parameters of the metabolites, M1 showed significant variations in each parameter after both treatments (Table 2); on the other side, the M2 and M5 predicted parameters showed no significant differences (data not shown).

Discussion

Nowadays, tramadol is widely used in veterinary clinical practice, despite its half-life being reported to be shorter in animals than in humans (Giorgi, 2008). The main active metabolite M1 has been shown to reach low plasma concentrations with a supposed lack of effectiveness in pain therapy (Giorgi *et al*., 2007, 2009a, b, d; de Sousa *et al*., 2008; Giorgi, 2008). In the present study, after the single oral dose of tramadol, the pharmacokinetic profiles of both tramadol and its main metabolites are in line with an earlier study carried out with reduced dosage (Giorgi *et al*., 2009d). Also, the wide variability found in the plasma concentrations of tramadol and its metabolites matches well with previous studies (Kukanich and Papich, 2004; Giorgi *et al*., 2009a, d). Once again, the present findings show a low M1 plasma concentration in dog.

In dog, GFJ has been used previously as lyophilized (Giorgi *et al*., 2003; Amatori *et*



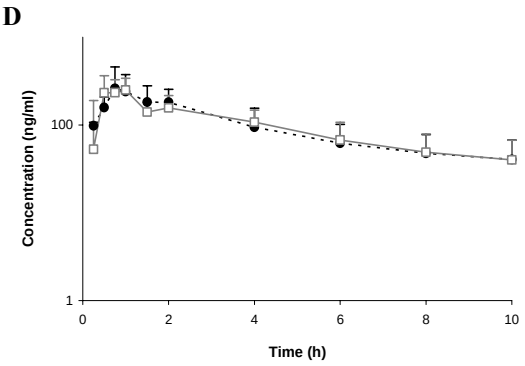


Fig. 2: A) Observed plasma concentration *vs* time of tramadol, following (●) single oral administration of tramadol (5 mg/kg) and (□) single co-administration of tramadol (5 mg/kg) and FD-GFJ (10 g/dog) to healthy Beagle dogs (n=6); B) Observed plasma concentration *vs* time of M1, following (●) single oral administration of tramadol (5 mg/kg) and (□) single co-administration of tramadol (5 mg/kg) and FD-GFJ (10 g/dog) to healthy Beagle dogs; C) Observed plasma concentration *vs* time of M2, following (●) single oral administration of tramadol (5 mg/kg) and (□) single co-administration of tramadol (5 mg/kg) and FD-GFJ (10 g/dog) to healthy Beagle dogs; D) Observed plasma concentration *vs* time of M5, following (●) single oral administration of tramadol (5 mg/kg) and (□) single co-administration of tramadol (5 mg/kg) and FD-GFJ (10 g/dog) to healthy Beagle dogs

al., 2004) and dry powder extract (Hanley *et al*., 2010) to increase the bioavailability of some drugs. According to previous studies (Giorgi *et al*., 2003; Amatori *et al*., 2004) 10 g of FD-GFJ have a similar effect than 100 ml of fresh liquid GFJ on the bioavailability of the drugs. Assuming that dogs and humans have the same metabolic pathway (Fig. 1), it has been speculated that the co-administration of FD-GFJ and tramadol might lead to an enhancement of M1 plasma concentration. Higher metabolisation rate of the CYP 2B and 3A than 2D are known in the dog; these enzymes could quickly metabolise either tramadol to M2, than M1 to M5, minimising the concentration of

Table 1: Predicted pharmacokinetic parameters of tramadol after single oral administration of tramadol (5 mg/kg) or co-administration of tramadol (5 mg/kg) and FD-GFJ (10 g), in six Beagle dogs

Parameters	Tramadol	FD-GFJ and tramadol
R^2	0.90±0.07	0.97±0.02
λz (1/h)	0.43±0.22	0.52±0.19
Hl λz (h)	1.90±1.00	1.45±0.42
T_{max} (h)	1.33±0.58*	1.70±0.45
C_{max} (µg/ml)	0.49±0.26*	0.27±0.21
$AUC_{0-\infty}$ (h·ng/ml)	2,060±1,630*	1,300±460
VZF (ml/Kg)	10,757±8,809	9,235±2,495
Cl/F (ml·kg/h)	6,134±3,326	4,473±1,418
$AUMC_{0-\infty}$ (h·h·ng/ml)	11,610±8,730	5,890±1,420
MRT (h)	3.76±0.98	3.35±1.42

*Significant value between the treatment groups ($P<0.05$). R^2 = correlation coefficient, λz = terminal phase rate constant, Hl λz = terminal half-life, T_{max} (h) = time of peak, C_{max} = peak plasma concentration, $AUC_{0-\infty}$ = area under the plasma concentration-time curve extrapolated to infinity, VZF = volume of distribution based on the terminal phase, Cl/F = clearance where F is the fraction of dose absorbed, $AUMC_{0-\infty}$ = area under the first moment curve from zero to infinity, and MRT = mean resident time

Table 2: Predicted pharmacokinetic parameters of M1 after single oral administration of tramadol (5 mg/kg) or co-administration of tramadol (5 mg/kg) and FD-GFJ (10 g), in six Beagle dogs

Parameters	Tramadol	FD-GFJ and tramadol
	M1	M1
R^2	0.95±0.06	0.98±0.02
λ (1/h)	0.41±0.08*	0.83±0.31
Hl λz (h)	1.74±0.31*	0.90±0.33
T_{max} (h)	1.36±0.88*	0.86±0.18
C_{max} (ng/ml)	80±70*	130±80
$AUC_{0-\infty}$ (h·ng/ml)	340±170*	160±140

*Significant value between the treatment groups ($P<0.05$). R^2 = correlation coefficient, λz = terminal phase rate constant, Hl λz = terminal half-life, T_{max} (h) = time of peak, C_{max} = peak plasma concentration, and $AUC_{0-\infty}$ = area under the plasma concentration-time curve extrapolated to infinity

M1 in plasma. This hypothesis agrees with the higher total V_{max} of 3A and 2B (0.35 ± 0.13 and 2.32 ± 0.92 nmol/min/mg protein, respectively), than of 2D (1.37 ± 0.95 nmol/min/mg protein), reported in Beagle dogs (Hojo *et al*., 2002), suggesting that the drug is metabolised faster from CYP 2B+3A than from CYP 2D. FD-GFJ is well-known to inhibit the CYP 3A intestinal enzymes and may indirectly promote the drug metabolism due to the CYP 2D, diverting more of the drug on this enzymatic way.

FD-GFJ has decreased the AUC, Cmax and the Tmax of tramadol, leading to a shift in the pharmacokinetic curve. The cause of this variation is unknown but could be related to the *in vitro* 2D6 inhibition caused by GFJ recently reported in one study (Girennavar *et al*., 2007). The FD-GFJ and tramadol co-administration has led to an increase in Cmax of M1, suggesting a reduction in CYP 3A activity and an enhancement in CYP 2D activity. Unfortunately the coincident reduction of AUC of M1 suggests a negligible effect. No differences were shown in the M2 and M5

parameters, speculating that CYP 3A could play a minor role in drug metabolism in the dog (Hojo *et al*., 2002).

To estimate the duration of action of both administrations, the clinically relevant therapeutic parameter Δt_e, the period of time during which minimum effective concentration (MEC) is exceeded, was calculated for an MEC. This research assumes that the MECs calculated in humans may also be effective in dogs. At MECs (calculated in humans) of 100 ng/mL and 40 ± 30 ng/mL, for tramadol and M1 (Lehmann *et al*., 1990), respectively, the Δt_e of T has been delayed from 0.16-4 h to 0.75-7 h after FD-GFJ and tramadol co-administration. On the contrary, the Δt_e of M1 has been advanced from 0.66-2.35 h to 0.1-1.75 h after the FD-GFJ and tramadol co-administration.

Finally, although FD-GFJ and tramadol co-administration has influenced the M1 plasma concentrations, they were well below the values reported in cats (Pypendop and Ilkiw, 2008) and humans (Lehmann *et al*., 1990). The present findings report this co-administration is not able to increase the M1 plasma concentrations to values close to those reported as effective in humans.

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