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Evaluation of the route dependency of the pharmacokinetics and neuro-pharmacokinetics of tramadol and its main metabolites in rats



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ABSTRACT

Tramadol hydrochloride is a centrally acting analgesic used for the treatment of moderate-to-severe pain. It has three main metabolites: O-desmethyltramadol (M1), N-desmethyltramadol (M2), and N,O-didesmethyltramadol (M5). Because of the frequent use of tramadol by patients and drug abusers, the ability to determine the parent drug and its metabolites in plasma and cerebrospinal fluid is of great importance. In the present study, a pharmacokinetic approach was applied using two groups of five male Wistar rats administered a 20 mg/kg dose of tramadol via intravenous (i.v.) or intraperitoneal (i.p.) routes. Plasma and CSF samples were collected at 5-360 min following tramadol administration. Our results demonstrate that the plasma values of C_{max} (C_0 in i.v. group) and area under the curve $(AUC)_{0-t}$ for tramadol were 23,314.40 \pm 6944.85 vs. 3187.39 \pm 760.25 ng/mL (C_{max}) and 871.15 \pm 165.98 vs. 414.04 \pm 149.25 µg · min/mL in the i.v. and i.p. groups, respectively (p < 0.05). However, there were no significant differences between i.v. and i.p. plasma values for tramadol metabolites (p > 0.05). Tramadol rapidly penetrated the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCSFB) $(5.00 \pm 0.00 \text{ vs. } 10.00 \pm 5.77 \text{ min in i.v. and i.p. groups, respectively})$. Tramadol and its metabolites (M1 and M2) were present to a lesser extent in the cerebrospinal fluid (CSF) than in the plasma. M5 hardly penetrated the CSF, owing to its high polarity. There was no significant difference between the AUC_{0-t} of tramadol in plasma $(414.04 \pm 149.25 \,\mu\text{g}\cdot\text{min/mL})$ and CSF $(221.81 \pm 83.02 \,\mu\text{g}\cdot\text{min/mL})$ in the i.p. group. In addition, the amounts of metabolites (M1 and M2) in the CSF showed no significant differences following both routes of administration. There were also no significant differences among the K_{p,uu,CSF(0-360)} (0.51 \pm 0.12 vs. 0.63 \pm 0.04) and K_{p,uu,CSF(0-∞)} $(0.61 \pm 0.10 \text{ vs. } 0.62 \pm 0.02)$ for i.v. and i.p. pathways, respectively (p > 0.05). Drug targeting efficiency (DTE) values of tramadol after i.p. injection were more than unity for all scheduled time points. Considering the main analgesic effect of M1, it is hypothesized that both routes of administration may produce the same amount of analgesia.

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1. Introduction

Drug delivery to the central nervous system (CNS) is considered to be a complicated process due to the presence of blood-brain and blood-cerebrospinal fluid barriers (i.e., BBB and BCSFB, respectively). In addition, various influx and efflux transporters exist throughout the

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CNS (Shen et al., 2004a). It is important to note that, in most cases, the plasma concentrations of centrally acting drugs cannot reflect their true brain concentrations. Therefore, it is crucial to measure the exposure of the brain to centrally acting drugs in order to predict their desired and unwanted central effects. It is obvious that only the drug molecule that is not bound to brain tissue (unbound) is available to interact with receptor sites, which is related to the pharmacological effects, or side effects, in the brain (Hammarlund-Udenaes, 2014). Consequently, unbound drug concentration in the brain interstitial fluid ($C_{u,brainISF}$) is the key parameter for the estimation of brain drug exposure. The brain/plasma ratio of unbound drug concentrations, known as $K_{p,uu,brain}$, has been particularly useful for understanding the extent of BBB transport. Intracerebral microdialysis has been used to measure $C_{u,brainISF}$, but it is not very common because of its experimental complexity, low throughput, and highly scattered data, particularly for

Abbreviations: BBB, blood-brain barrier; AUCO-360 min, area under the curve to 360 min; AUCO-∞, area under the curve to time infinity; BCSFB, blood-cerebrospinal fluid barrier; Cmax, maximum concentration; CSF, cerebral spinal fluid; CCSF, CSF concentration; CNS, central nervous system; HPLC, high-performance liquid chromatography; ISF, interstitial fluid; i.p., intraperitoneal; i.v., intravenous; MRT, mean residence time; Tmax, time to reach Cmax.

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lipophilic compounds (Westerhout et al., 2011). The closeness of the cerebrospinal fluid (CSF) to the brain and its relationship with brain interstitial fluid (ISF) have led to consideration of the drug concentration in the CSF (CCSF) as a practical surrogate for C_{u,brain}. Xenobiotics and drugs within the circulatory system can reach the CSF either via direct passage across the choroid plexus, or indirectly through diffusion transport across the BBB from the interstitial fluid to the CSF. In humans, the CSF is the best accessible alternative tool to determine unbound brain concentrations (Shen et al., 2004b; Lin, 2008; De Lange and Danhof, 2002). In addition, CSF is a readily accessible biological matrix in preclinical studies with a sampling procedure less predisposed to experimental requirements compared to the microdialysis technique. Moreover, the possibility of consecutive CSF sampling through catheters inserted into the cisterna magna or lumbar intrathecal space makes the CSF a comprehensive guide to the time course of drug concentrations in the CNS.

Tramadol hydrochloride [*trans*-(\pm)-2-[(dimethylamino)methyl]-1-(3-meth-oxyphenyl)cyclohexanol, hydrochloride (Fig. 1)] is a centrally acting synthetic opioid that has antinociceptive and analgesic effects in animals and humans (Raffa, 2008). It also exhibits monoaminergic activity produced by the inhibition of norepinephrine and serotonin reuptake in synaptic junctions (Raffa et al., 1992). Tramadol is extensively metabolized in the liver by hepatic *O*- and *N*-demethylation pathways catalyzed by the cytochrome P-450 isoenzymes 2D6, 2B6, and 3A4, in that order (Emerson and Pope, 1999). Of these, *O*-desmethyltramadol (M1) and *N*-desmethyltramadol (M2) are the main metabolites of tramadol in humans, while M1 and M2 may further be metabolized to produce *N*,*O*-didesmethyltramadol (M5) and other metabolites. Among the tramadol metabolites, M1 represents the primary active metabolite; M5 is also an active metabolite, but has weaker analgesic activity compared with M1 (García-Quetglas et al., 2007; Wu et al., 2002).

The aim of this study was to assess the neuropharmacokinetic properties of tramadol and its main metabolites in the CSF, and to investigate the impact of administration route on these parameters. The effects of administration route (i.e., intravenous, intraperitoneal, or oral) on the stereoselective pharmacokinetics of tramadol and M1 have been previously studied in rats via blood and urine analysis (Parasrampuria et al., 2007); however, M2 and M5 metabolites have not evaluated. Indeed, for the first time, we have quantitatively analyzed the pharmacokinetic differences of tramadol and its main metabolites (M1, M2, and M5) in CSF and plasma following intravenous and intraperitoneal tramadol administration to male rats. Only a few reports in the literature have evaluated the neuropharmacokinetics of tramadol. The pharmacokinetic properties of tramadol in rat plasma and CSF were reported in a study after intranasal, intravenous, and oral administration (Zhao et al., 2008). Wang et al. (2015) also studied the presence of tramadol and M1 in the CNS, investigating changes in the pharmacokinetic and pharmacodynamic properties of tramadol after co-administration with both brain CYP2D inducers and inhibitors. In addition, concentration-time profiles of tramadol and M1 in rat CSF and plasma were evaluated following intraperitoneal injection (40 mg/kg tramadol) (Wang et al., 2015).

Fig. 1. Chemical structure of tramadol.

2. Materials and methods

2.1. Materials and animals

Tramadol hydrochloride and its metabolites, including M1, M2 and M5, as well as *cis*-tramadol (internal standard) were supplied by Grünenthal (Stolberg, Germany). HPLC-grade acetonitrile and methanol and analytical grade ethyl acetate and phosphoric acid (85%) were purchased from Merck (Darmstadt, Germany). All other chemicals and solvents were of analytical grade and used without further purification.

Adult male Wistar rats weighing between 250 and 300 g and aged between 75 and 90 days were housed in standard cages in a temperature- and humidity-controlled room with a 12-h light–dark cycle and access to water and standard rat chow ad libitum. The whole animal study protocol was approved by the Institutional Review Board of the Pharmaceutical Research Centre of Tehran University of Medical Sciences, Iran (Code of ethics: 9411392003).

2.2. Drug administration and sampling

Rats were generally anesthetized via an intraperitoneal (i.p.) injection of a ketamine-xylazine mixture (ketamine 100 mg/kg and xylazine 10 mg/kg). The right external jugular veins were catheterized using a rubber catheter made of two pieces of polyethylene [a 10-cm piece of polyethylene (PE-50) tubing (I.D. 0.023 in. \times 0.D. 0.038 in., wall 0.008 in.)] and silicone rubber tubing [a 2.5–3-cm piece of medical grade silastic tubing (I.D. 0.025 in. \times 0.D. 0.047 in., wall 0.011 in.)] according to a standard surgical procedure (Waynforth and Flecknell, 1992). Animals were then housed separately and left overnight for complete recovery, before being anesthetized the next day with an i.p. injection of the above mentioned mixture. Rats were then fixed onto a stereotaxic apparatus in order to cannulate their cisterna magna with a 25-gauge needle attached to a 10 cm polyethylene tube [polyethylene (PE-50) tubing (I.D. 0.023 in. × O.D. 0.038 in., wall 0.008 in.)] (Mahat et al., 2012). Following the control of the cannula inserted in the cisterna magna, animals were administered a dose of tramadol hydrochloride (20 mg/kg of body weight) dissolved in a saline-ethanol vehicle (1:1 v/v) via gavage, intravenous, or intraperitoneal injection. Blood and CSF samples were collected via the catheters at predetermined sampling time points (5, 15, 30, 60, 120, 240, 300, and 360 min following tramadol administration), with a volume of 300-µL blood and 20-µL CSF withdrawn each time. Sterile 0.9% saline was used in order to compensate the blood loss with the volume of fluid replacement equivalent to the volume of blood drawn. Plasma samples were collected in heparinized tubes. Blood samples were centrifuged at 1000 g for 10 min to separate the plasma, and all samples were kept frozen at -20 °C until drug analysis. Five rats were used in each group to collect plasma and CSF samples.

2.3. Bioanalytical procedures

In order to determine the concentration of tramadol and its metabolites (M1, M2, and M5) in plasma and CSF samples, a reversed phase high performance liquid chromatography (HPLC) method, combined with a liquid-liquid extraction step was used (Ardakani and Rouini, 2007). Briefly, 50 μ L NaOH (2 N) and 50 μ L of internal standard (*cis*-tramadol, 1 μ g/mL) aqueous solutions were added to 120 μ L of plasma and the mixture was vortexed for 30 s. To extract the analytes from the plasma matrix, 1.2 mL ethylacetate was added. The resulting mixture was shaken for 15 min and then centrifuged at 10,000 g for 10 min to separate the organic layer, which was transferred to a clean glass tube and evaporated to dryness under a gentle air stream at 50 ° C. As a final step, 120 μ L of mobile phase was added to the residue from evaporation (to reconstitute the samples) and 100 μ L of each sample was injected into the HPLC. CSF samples were directly injected to the chromatograph.

Analytes were separated using a mobile phase consisting of methanol and water (adjusted to pH 2.5 with phosphoric acid; 19:81 v/v) delivered in isocratic mode at a flow rate of 2 mL/min onto a ChromolithTM Performance RP-18e column (100 mm \times 4.6 mm) (Merck, Darmstadt, Germany). The column was protected by a ChromolithTM RP-18e Guard Cartridge (5 mm \times 4.6 mm) (Merck, Darmstadt, Germany). The detection was carried out by a fluorescence detector set at excitation and emission wavelengths of 200 and 301 nm, respectively.

The chromatographic instrument (Knauer, Berlin, Germany) consisted of a double-reciprocating delivery system, a fluorescence detector, and an online degasser. A Rheodyne injector (model 7725i) equipped with a 100 μ L loop was used for sample injection. Chromatographic data acquisition was performed using the compatible software (Knauer, ChromGate, Berlin, Germany). The mentioned bioanalytical method provided an accuracy of 89.3–106.7% and precision of better than 12.7% for control samples. Chromatograms of tramadol, M1, M2, M5 in the plasma (A) and CSF (B) samples are shown in Fig. 2.

Unknown samples were quantitated against calibration curves for plasma and CSF prepared by the addition of 25–1000 ng/mL of tramadol and its metabolites to plasma and Krebs buffer, respectively.

2.4. Pharmacokinetic analysis

A non-compartmental model was used to determine the plasma and CSF pharmacokinetic parameters of tramadol and its metabolites (M1, M2, and M5). The maximum concentration (C_{max}), time to reach Cmax (T_{max}), area under the curve to 360 min (AUC_{0-360 min}), area under the curve to time infinity (AUC_{0-∞}), mean residence time (MRT), and half-life ($t_{1/2}$) of the analytes were determined using the PKSolver software program (Zhang et al., 2010). Data were presented as mean \pm standard deviation (SD). The relationship of tramadol, M1,

and M2 between the plasma and CSF was evaluated by linear regression analysis.

The unbound partition coefficient of tramadol in CSF ($K_{p,uu,CSF}$) was obtained as follows (Fridén et al., 2009; Chen et al., 2014):

$$K_{p,uu,CSF} = \frac{AUCu, CSF}{AUCu, Plasma}$$
(1)

Where $AUC_{u,CSF}$ is the area under the unbound CSF concentrationtime curve, and $AUC_{u,Plasma}$ is the area under the unbound plasma concentration-time curve.

$$C_{u,Plasma} = C_{Plasma} \times f_{u,Plasma}$$
(2)

where: $C_{u,Plasma}$ is the unbound plasma concentration, C_{Plasma} is the plasma concentration, and $f_{u,Plasma}$ is the fraction of unbound drug in plasma.

$$C_{u,CSF} = C_{CSF} \times f_{u,CSF} \tag{3}$$

Where $C_{u,CSF}$ is the unbound CSF concentration, C_{CSF} is CSF concentration, and $f_{u,CSF}$ is the fraction of unbound drug in CSF.

$$f_{u,CSF} = \frac{1}{1 + Q_{alb} \left(\frac{1}{fu, p} - 1\right)}$$
(4)

 Q_{alb} in Eq. (4), which presents the albumin CSF to plasma ratio, was considered as 0.003 for rat CSF samples originating from the cisterna magna (Narang et al., 1988; Hoetelmans et al., 1997), while 0.85 for the unbound fraction ($f_{u,plasma}$) of tramadol was used (Fridén et al., 2009).



Fig. 2. Chromatograms of tramadol (T) and its metabolites (M1), (M2), (M5) in rat plasma and CSF. (A) Blank rat plasma spiked with IS (*cis*-tramadol). (B) Blank rat CSF (C) Plasma sample of a rat 2 h following a 20 mg/kg intravenous dose of tramadol (D) CSF sample of a rat 2 h following a 20 mg/kg intravenous dose of tramadol.

The brain targeting of tramadol following the intraperitoneal route was also evaluated by calculating drug targeting efficiency (DTE) (Zhao et al., 2008):

$$DTE = \frac{\left(\frac{AUCu, CSF}{AUCu, Plasma}\right)I.P}{\left(\frac{AUCu, CSF}{AUCu, Plasma}\right)I.V}$$
(5)

2.5. Statistical analysis

The statistical non parametric Mann-Whitney test at a significance level of 0.05 was applied in order to analyze the differences between the data sets (the intravenously drug administered group vs. the intraperitoneally drug administered group). The data are presented as mean \pm SD.

3. Results

Results from the oral group are not discussed here because of observed fluctuations related to incomplete absorption in anesthetized rats.

The total plasma and CSF concentration-time courses following intravenous or intraperitoneal administration (20 mg/kg) of tramadol are presented in Fig. 3A, B, C, and D for tramadol, M1, M2, and M5, respectively. A summary of model-independent plasma and CSF pharmacokinetic parameters of tramadol and its metabolites is shown in Tables 1 and 2.

Tramadol was quickly absorbed in circulation following intraperitoneal administration and reached its peak concentration (3187.39 \pm

760.25 ng/mL) after approximately 10 min (Table 1). The major metabolites, M1, M2, and M5, were concurrently detected in both plasma and CSF samples. The process of metabolism after either route of administration quickly started and caused M1 and M5 to show their maximum plasma concentrations in <30 min. However, M2 showed greater variation among the animals and it took longer to reach its peak plasma concentration (122.00 \pm 109.58 vs. 116.25 \pm 111.16 min in i.v. and i.p. groups, respectively). As expected, values of C_{max} (C_0 in i.v. group) and AUC_{0-t} in plasma for tramadol after intravenous administration were significantly different than those after intraperitoneal injection (23,314.40 \pm 6944.85 vs. 3187.39 \pm 760.25 ng/mL and 871.15 \pm 165.98 vs. 414.04 \pm 149.25 µg \cdot min/mL in i.v. and i.p. groups, respectively p < 0.05). However, there was no significant difference between the values for metabolites in plasma (p > 0.05). In fact, higher, but not significant, concentrations were observed for M2 and M5 following the intraperitoneal injection compared with those of i.v. injection. In the case of M1, calculated AUC_{0-t} values were 459.45 \pm 274.51 vs. 306.93 \pm 70.57 ng·min/mL following intravenous and intraperitoneal administration, respectively (p > 0.05). However, it took longer to reach M1 peak plasma concentration in the i.p. group compared with the i.v. group (18.75 \pm 7.50 min in i.p. group vs. 9.00 \pm 5.48 min in i.v. group (p < 0.05).

Tramadol rapidly penetrated the BBB and BCSFB and reached the CSF following intravenous injection (5.00 ± 0.00 vs. 10.00 ± 5.77 min in i.v. and i.p. groups, respectively). The T_{max} of tramadol in CSF in both groups was longer than that in the plasma but the difference was not significant following intravenous administration (p > 0.05). Early detection of M1



Fig. 3. Plasma and CSF concentrations (ng/ml) of T (A), M1 (B), M2 (C) and M5 (D) following a 20 mg/kg intravenous and intraperitoneal dose of tramadol (mean ± SE, n = 5).

Table I

Parameters		C _{max} (ng/ml)	T _{max} (min)	AUC_{0-t} (µg·min/ml)	T _{1/2} (min)	MRT (min)
Tramadol	Plasma	$23{,}314{.}40\pm6944{.}85^*$	-	871.15 ± 165.98	182.04 ± 47.58	103.68 ± 8.72
	CSF	4458.41 ± 947.50	5.00 ± 0.00	380.76 ± 33.59	238.20 ± 127.96	115.34 ± 11.52
M1	Plasma	2526.94 ± 1358.62	9.00 ± 5.48	459.45 ± 274.51	335.85 ± 164.00	151.56 ± 15.16
	CSF	575.71 ± 83.59	21.00 ± 8.22	137.48 ± 10.82	332.11 ± 104.96	158.25 ± 11.89
M2	Plasma	844.51 ± 330.33	122.00 ± 109.58	207.99 ± 90.37	-	166.60 ± 22.24
	CSF	275.94 ± 75.04	264.00 ± 32.86	78.33 ± 19.23	-	184.38 ± 9.74
M5	Plasma	299.44 ± 94.47	21.00 ± 8.22	67.97 ± 12.80	_	163.45 ± 7.38
	CSF	25.75 ± 12.01	210.00 ± 134.16	5.61 ± 2.27	-	180.46 ± 32.86

Pharmacokinetic parameters of tramadol and its metabolites following intravenous administration of a 20 mg/kg dose of tramadol (r	$(mean \pm SD, n = 5)$	1)
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* The reported value is the C₀ of tramadol in plasma.

was also seen in the CSF following tramadol administration in both groups ($T_{max} < 1$ h). The distribution rate of M2 in CSF followed the same trend as in plasma (longer and more variable T_{max} compared with that of M1). Insignificant M5 concentrations in the CSF were observed.

Similar to results in plasma, rats in the intravenous group had comparable C_{max} , $AUC_{0-360 \text{ min}}$, and $MRT_{0-360 \text{ min}}$ values for M1 and M2 in the CSF to those in the intraperitoneal group. However, there was a delay in reaching maximum concentrations of M1 in the CSF following the intraperitoneal tramadol dose (p < 0.05) (Tables 1 and 2).

The MRT_{0-t} values for all metabolites were significantly longer than those for tramadol in both plasma and CSF samples (p > 0.05).

Relationships between plasma and CSF concentrations of tramadol and its metabolites (M1, M2) in both groups of intravenous and intraperitoneal administration are presented in Fig. 4. According to Pearson correlation coefficients for tramadol, M1, and M2, which are shown in Table 3, there appears to be a very strong linear correlation between CSF and plasma concentrations of tramadol (>0.8), while M1 concentrations in the CSF and plasma were only low to moderately correlated. In the case of M2, a moderate to strong linear correlation was seen between CSF and plasma concentrations.

To further pursue metabolism behavior, metabolite ratios were studied for M1 and M2. The metabolite ratios $(AUC_{0-t} Metabolite/AUC_{0-t} Tramadol)$ for M1 and M2 in both the plasma and CSF are shown in Fig. 5. Calculated values for both metabolites in plasma show significant differences between the two intravenous and intraperitoneal injection groups. However, there was no significant difference between metabolite ratios of each metabolite in CSF following i.p. or i.v. administration.

The amount of $K_{p,uu,CSF}$ for tramadol was calculated following both routes of administration. There were no significant differences among the $K_{p,uu,CSF(0-360)}$ (0.51 \pm 0.12 vs. 0.63 \pm 0.04) and $K_{p,uu,CSF(0-\infty)}$ (0.61 \pm 0.10 vs. 0.62 \pm 0.02) for i.v. and i.p. pathways, respectively (p > 0.05).

DTE values of tramadol after i.p. injection for all scheduled time points are presented in Table 4; all calculated values were higher than unity.

4. Discussion

To our knowledge, the present paper is the first study investigating tramadol and its metabolites in plasma and cerebrospinal fluid following different administration routes in male Wistar rats. Despite the presence of a few studies in the literature (Parasrampuria et al., 2007; Valle et al., 2000; Garrido et al., 2003; Zhao et al., 2008), simultaneous investigation of the pharmacokinetics of tramadol and its main metabolites in rat plasma and CSF has not been undertaken. The pharmacokinetic-pharmacodynamic relationship of (+)-tramadol and its two main metabolites, (+)-O-desmethyltramadol and (-)-Odesmethyltramadol, have, however, been studied in rats (Valle et al., 2000; Garrido et al., 2003); pharmacokinetics of tramadol and both enantiomers of M1 were described using compartmental models in previous studies. The effect of CYP2D activity on the pharmacokinetics of these compounds was also investigated, along with the time course of response and antinociceptive effects of the parent drug and M1 enantiomers. However, earlier work did not quantify the amount of tramadol and its metabolites in the CNS (Valle et al., 2000; Garrido et al., 2003). this study, we attempted to carry out a complete In neuropharmacokinetic study to describe the quantitative distribution of this opioid-like drug and its metabolites in the CSF as an available surrogate for interstitial fluid, with a focus on the effect of administration route.

The HPLC method used throughout this study was developed in our lab by Ardakani and colleagues (Ardakani and Rouini, 2007) for drug assays in human plasma, urine, and saliva and was also applied to rat plasma and brain (Sheikholeslami et al., 2012). We have also adopted this method for drug assays in CSF samples. However, as CSF is not available in adequate quantities, analytical method validation was performed using the Krebs-Henseleit buffer instead of CSF. The sensitivity was suitable in terms of detection and quantitation of low concentration samples throughout the study. Method validation data was excluded from this article in the interest of manuscript length.

By considering the complexity and difficulties of microdialysis, CSF was used as the best informative tool for unbound brain drug exposure in this study. As mentioned earlier, CSF drug concentration has been considered the best possible surrogate for unbound drug concentrations in brain (C_u). However, the closeness of CSF concentrations to brain ECF concentrations in different regions in the brain, substrates of efflux drug transporters (P-gp and BCRP), and some pathological conditions has raised concerns (De Lange and Danhof, 2002; Fridén et al., 2009; Kodaira et al., 2011). In the case of tramadol, Fridén et al. (2009) showed that CSF concentrations under-predict ISF concentrations. They related the differences between the drug concentration in these matrices to

Table 2

Pharmacokinetic parameters of tramadol and its metabolites following intraperitoneal administration of a 20 mg/kg dose of tramadol (mean \pm SD, n = 5).

Parameters		C_{max} (ng/ml)	T _{max} (min)	AUC_{0-t} (µg·min/ml)	T _{1/2} (min)	MRT (min)
Tramadol	Plasma	3187.39 ± 760.25	10.00 ± 5.77	414.04 ± 149.25	141.87 ± 25.73	127.45 ± 6.46
	CSF	1520.01 ± 500.93	26.25 ± 7.50	221.81 ± 83.02	126.14 ± 26.20	119.87 ± 10.84
M1	Plasma	1765.99 ± 112.25	18.75 ± 7.50	306.93 ± 70.57	303.45 ± 84.44	151.72 ± 12.31
	CSF	533.44 ± 56.71	45.00 ± 17.32	124.58 ± 26.95	262.20 ± 85.29	159.97 ± 13.50
M2	Plasma	1011.24 ± 300.09	116.25 ± 111.16	252.18 ± 86.57	_	177.56 ± 15.79
	CSF	344.61 ± 88.45	165.00 ± 141.77	91.35 ± 17.57	_	183.26 ± 10.87
M5	Plasma	370.60 ± 76.21	26.25 ± 7.50	88.89 ± 30.27	_	165.56 ± 16.07
	CSF	36.63 ± 13.86	270.00 ± 142.83	7.34 ± 3.13	-	197.63 ± 57.40



Fig. 4. The relationships between the tramadol M1 and M2 concentrations in the plasma and CSF following intravenous (3 A, 3 B, 3C) and intraperitoneal (3 D, 3E, 3 F) administration of a 20 mg/kg dose of tramadol.

Table 3

The relationships between the tramadol or its metabolites (M1, M2) concentrations in the plasma and CSF for both groups of intravenous and intraperitoneal administration

Analytes	Pearson coeff	Pearson correlation coefficient i.v. i.p.		ficance
	i.v.			i.p.
Т	0.89	0.87	0.00	0.00
M1	0.24	0.62	0.09	0.00
M2	0.60	0.73	0.00	0.00



Fig. 5. Comparison of metabolite ratios for M1 and M2 in plasma and CSF between groups of intravenous and intraperitoneal administration of a 20 mg/kg dose of tramadol (* and **: significant difference, p < 0.05).

the dissimilarities in the expression or function of organic cation transporters (OCTs) at the BBB and BCSFB (Fridén et al., 2009). However, an in vitro uptake study using hCMEC/D3 cells highlighted the role of the H + /OC antiporter in the active transport of tramadol through the human BBB (Kitamura et al., 2014).

As mentioned earlier, we did not evaluate the results of the oral group because of observed inter-animal variation related to gastrointestinal function and incomplete drug absorption in anesthetized rats. Indeed, Torres-Molina et al. (1996) also found that heavy anesthesia in rats can significantly alter drug absorption following oral administration of amoxicillin.

According to our results, the observed plasma concentrations of tramadol following intraperitoneal administration were significantly lower than those of intravenous administration because of higher levels of tramadol metabolism, the result of direct drug entrance to the portal vein via the intraperitoneal route.

As expected, in both intravenous and intraperitoneal routes, the areas under the plasma concentration-time curves $(AUC_{0-\infty} \text{ and } AUC_{0-360})$ for tramadol and its metabolites were significantly higher than the area under the CSF concentration-time curves. As we used CSF to determine the unbound concentrations in the present study, lower concentrations were seen compared with our previous study in brain homogenate. High polarity of the M1, M2, and M5 metabolites caused lower concentrations of these compounds in the CSF.

with the results of a previous study (Kogel et al., 1999) based on the inability of M5 to penetrate the BBB, despite the significant concentrations of this metabolite in plasma regardless of administration route, in this study very negligible amounts of M5 were observed in the CSF.

The results of our linear regression analysis, between plasma and CSF concentrations of tramadol and its metabolites, are in agreement with the results of a previous study conducted by Wang et al. (2015). However, these researchers only investigated the relationship between plasma and CSF concentrations of tramadol and M1. The findings of the present study suggest that, similar to those reported for tramadol (Wang et al., 2015), the majority of M2 concentrations in the CSF may be linked to the influx of this metabolite from the plasma. However, M1 behavior was different with tramadol and M2. Similar to the results reported by Wang et al. (2015), M1 displayed a low-to-moderate regression coefficient in our study, suggesting that M1 levels in the CSF, following both routes of tramadol administration, could not be only related to influx phenomena form plasma. This could be due to two possible mechanisms; one is in situ metabolism of tramadol via brain CYP2D, as hypothesized by Wang et al. (2015), and supported by the results of Miksys and Tyndale (2009), indicating the presence of active drug metabolizing enzymes in the brain when investigating codeine metabolism via in situ brain CYP2D (Zhou et al., 2013). The second mechanism may be related to the presence of organic cation transporters at the BBB as reported by Tzvetkov et al. (2011). Organic cation transporters (OCTs) are categorized as a subfamily of influx transporters involved in the distribution and excretion of their substrates (Koepsell et al., 2007). Organic cations and weak bases that are positively charged at physiological pH are the main substrates of OCTs. However, they can also transport some non-charged molecules (Koepsell et al., 2007).

As shown in Fig. 3 and Tables 1 and 2, there was no significant difference between the area under the plasma and CSF concentration-time curves of M1, the major metabolite of tramadol, after intravenous or intraperitoneal tramadol administration (p > 0.05). The same trend has been observed for M2 (p > 0.05). According to the main analysic effect of M1, and based on these findings, we hypothesize that the efficacy of tramadol may be independent of the route of administration. As mentioned, in order to more precisely investigate the metabolism trend, the metabolite ratios in plasma and CSF were studied for both M1 and M2. According to the results for both metabolites, the metabolite ratios in the CSF between the two groups showed no significant differences. This evidence also implies the possible existence of the above mentioned mechanisms influencing the presence of metabolites in the CSF. The possible involvement of brain metabolizing enzymes should be taken into account in this case. These findings are in agreement with the results of the effect of tramadol on micturition in rats (Pandita et al., 2003). Indeed, (\pm) Tramadol effectively inhibits micturition in conscious rats by stimulating µ-opioid receptors and monoamine reuptake inhibition (Anderson, 1993; De Groat and Yoshimura, 2001; De Groat, 2002). Pandita et al. (2003) showed that the effect of i.p. administration of tramadol in a dose of 5 mg/kg on threshold and micturition pressure, as well as micturition volume, were not significantly different from those after i.v. administration. The similarity in tramadol effects following both routes of administration might be due to the same concentrations of M1 on target receptors, also were seen in the present study.

In order to calculate the $f_{u,CSF}$ and $K_{p,uu,CSF}$, we used the $f_{u,plasma}$ of 0.85 for tramadol reported by Fridén et al. (2009). Thus, $f_{u,CSF}$ was 0.99 in our study, indicating that the amount of tramadol protein binding

Table 4

K_{p,uu,CSF} during the time (0-t) following intravenous and intraperitoneal administration of a 20 mg/kg dose of tramadol and Drug targeting efficiency following intraperitoneal administration of a 20 mg/kg dose of tramadol

Time (min)	5	15	30	60	120	180	240	300	360
K _{p,uu,CSF (IV)} K _{p,uu,CSF (I.P.)} DTE	$\begin{array}{c} 0.14 \pm 0.04 \\ 0.33 \pm 0.06 \\ 2.31 \end{array}$	$\begin{array}{c} 0.30 \pm 0.07 \\ 0.44 \pm 0.09 \\ 1.47 \end{array}$	$\begin{array}{c} 0.39 \pm 0.08 \\ 0.56 \pm 0.11 \\ 1.44 \end{array}$	$\begin{array}{c} 0.45 \pm 0.10 \\ 0.64 \pm 0.11 \\ 1.42 \end{array}$	$\begin{array}{c} 0.50 \pm 0.12 \\ 0.68 \pm 0.07 \\ 1.35 \end{array}$	$\begin{array}{c} 0.52 \pm 0.11 \\ 0.67 \pm 0.06 \\ 1.30 \end{array}$	$\begin{array}{c} 0.52 \pm 0.11 \\ 0.66 \pm 0.06 \\ 1.25 \end{array}$	$\begin{array}{c} 0.53 \pm 0.12 \\ 0.64 \pm 0.05 \\ 1.21 \end{array}$	$\begin{array}{c} 0.51 \pm 012 \\ 0.63 \pm 0.04 \\ 1.23 \end{array}$

in CSF is negligible. The values of K_{p,uu,CSF} for tramadol in the current study following both routes of administration were in good agreement with the results of the study presented by Fridén et al. (2009), who also reported a K_{p,brain} of 5.29 for tramadol in rats. In our previous study, we used a $K_{p(1 \text{ min})}$ of tramadol in rat brain homogenates (Sheikholeslami et al., 2012). Considering that the $K_{p(1 \text{ min})}$ values from the mentioned study ranged from 2.47 to 3.50 in a dose range of 1 to 10 mg/kg, the K_{p,brain} of 5.29 by Fridén et al. (2009), and the results of the current study, it is obvious that total brain to plasma concentration ratio is not reflecting the proper CNS distribution of tramadol. Our previous study showed that tramadol accumulates in brain. The brain concentrations of tramadol in that study were significantly higher than its plasma concentrations because of nonspecific binding to brain proteins and also intracellular distribution of drug. Indeed, the presence of the BBB, BCSFB, and several processes, including the passive and active uptake and efflux at the BBB, distribution to the brain cells, specific and nonspecific binding, and entering to the organelles, altogether influence drug equilibration in the CNS (Hammarlund-Udenaes, 2014). It has been shown that basic drugs accumulate in low-pH parts. The pH of blood is 7.4, while that of the CSF and brain ISF are approximately 7.33 and 7.3, respectively; pH of the cell cytosol is 7.0 (Hammarlund-Udenaes, 2014). These pH differences also influence equilibration of tramadol as a basic drug (pKa = 9.7) in different compartments of the CNS. Tramadol accumulation in brain due to the above mentioned reasons causes the K_{p,brain} values to be much higher than 1. The K_{p,uu,CSF} is smaller than unity according to the results of our study and also previous reports. As there was no information relating to the protein binding of metabolites, we did not calculate the K_{p.uu.CSF} for metabolites.

In order to evaluate the ability of the intraperitoneal route to pass tramadol into the CNS, DTE was calculated. DTE values larger than unity would suggest a higher capability of an administration route compared with the intravenous administration to pass the drug into the CNS. In the present study, following the intraperitoneal administration, all DTE values from 0 to 360 min were >1 (Table 4). According to the descending trend of the DTE values with time (from 2.31 to 1.23), the reason of the higher values can be related to the very high plasma concentrations of tramadol following the intravenous administration, especially during the earlier time points. In other words, the bioavailability of the intraperitoneal route for tramadol, which was around 50% in the present study, caused the significant differences between the plasma concentrations of tramadol in the two groups of rats and a consequent increase in DTE values. However, if we exclude the values of the 0–5 min interval from the calculation of DTE and K_{p.uu.CSF}, all the DTE values for intraperitoneal administration will be near unity and the differences among the K_{p,uu,CSF} following both routes of administration during the time will be insignificant.

5. Extrapolations of the results to humans

The biotransformation pattern of tramadol has been shown to be almost qualitatively identical in humans and rats. In both species, M1, M2, M5, and conjugates of both M1 and M5 are the main metabolites. However, tramadol is metabolized much more rapidly in animals than in humans, which results in considerable differences between the amounts of urinary excretion of unchanged tramadol (Lintz et al., 1981). In addition, Parasrampuria et al. (2007) suggested that the rat can be considered as a suitable model for enantioselective studies of tramadol pharmacokinetics in human. They found an agreement between the direction and extent of stereoselectivity in the pharmacokinetics of tramadol and its main metabolite, M1, in rats and humans (Parasrampuria et al., 2007). In another report, predicted average steady state concentrations of M1 relative to humans were shown to be 60% in rats (Holford et al., 2014). Moreover, the H+/OC antiporter, which played a major role in the active transport of tramadol across the human BBB in an in vitro uptake study conducted by Kitamura et al. (2014) using hCMEC/D3 cells, showed good similarity between rat and human brain endothelial cells (TR-BB13 and hCMEC/D3 cells, respectively) (Shimomura et al., 2013; Kitamura et al., 2014;). According to these previous reports, which focused on the observed similarities in tramadol metabolism and transport between humans and rats, the results of this study may be used for initial predictions of the neuropharmacokinetics of tramadol in humans.

6. Conclusion

In summary, we have provided the first direct evidence for the differences between CSF uptake of tramadol and two of its metabolites, M1 and M2, following intravenous and intraperitoneal administration routes. By considering the same amount of M1 in the CSF following both i.v. and i.p. administration, and also its main analgesic effect, it appears that both routes of administration may produce the same amount of analgesia.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All procedures performed in studies involving animals were in accordance with the ethical standards of the Animal Ethic Committee and Institutional Review Board of Pharmaceutical Research Centre of Tehran University of Medical Sciences.

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