Pharmacokinetic study of tramadol and its three metabolites in plasma, saliva and urine

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ABSTRACT

Background and the purpose of the study: Pharmacokinetic parameters of tramadol and its three metabolites in plasma, saliva and urine following administration of 100 mg single oral dose were investigated in 24 healthy volunteers.

Materials and Methods: 12 male and 12 female healthy volunteers received a single oral dose of tramadol and Plasma, mixed saliva -secreted samples without any stimulation and urine were analyzed for Tramadol and its main metabolites by HPLC method.

Results and Disscusion: Almost 16.2% of tramadol and 11.2, 1.1 and 5.0% of *O*-desmethyltramadol (M1), *N*-desmethyltramadol (M2) and *N,O*-didesmethyltramadol (M5) respectively were recovered in 30 hrs collected urine. Renal clearance of tramadol, M1, M2 and M5 were 114.7 ± 44.5, 193.9 ± 67.6, 116.1 ± 61.8 and 252.0 ± 91.5 (mL/min) respectively. The maximum plasma concentration of tramadol, M1, M2 and M5 were 349.3 ± 76.7, 88.7±30.3, 23.1 ± 11.4 and 30.0 ± 11.7 (ng/mL) at 1.6 ± 0.4, 2.4 ± 0.7, 2.8 ± 1.0 and 2.7 ± 1.4 hrs after drug administration respectively. Tramadol and its metabolites appeared in a significant amount in saliva with the saliva/plasma ratios of 9.0, 1.6, 12.3 and 2.8 for tramadol, M1, M2 and M5 according to $AUC_{(0-24)}$ respectively.

Conclusion: Conclusion Strong correlations were found between plasma and saliva concentrations for all studied compounds and a dissection to pre and post absorption components improved these correlations. Results o f this study suggests that saliva is a suitable alternative to plasma for clinical and toxicological studies of tramadol and in addition to passive diffusion, a possible active transport is also suggested to describe the elevated saliva/plasma ratios for these compounds.

Keywords: Tramadol, Metabolite, Saliva, Urine, Pharmacokinetic

INTRODUCTION

Tramadol hydrochloride (T), a synthetic 4-phenyl-piperidine analogue of codeine, is a centrally acting analgesic with efficacy and potency ranging between weak opioids and morphine. Tramadol produces analgesia synergistically combined with weak μ-opioid and monoaminergic (noradrenaline and serotonin) mediated mechanisms (1).

Tramadol is rapidly and almost completely absorbed after oral administration. However, its mean absolute bioavailability is only 65–70% due to the first-pass hepatic metabolism. Following a single 100 mg oral dose, plasma C_{max} of approximately 300 ng/mL is reached within 1-3 hrs after administration (2).

Tramadol is distributed in the body, with a mean distribution half-life of 1.7 hrs. The high total distribution volume of 306 liters (L) after oral administration indicates its high tissue affinity. The plasma protein binding of this drug is reported about 20% (3).

This compound is rapidly and extensively metabolized in the liver by two principal pathways: O-demethylation to O-desmethyltramadol (M1) by CYP2D6 and N-demethylation to N-desmethyltramadol (M2) by CYP2B6 and CYP3A4. The primary metabolites; O-desmethyltramadol (M1) and N-desmethyltramadol (M2) may be further metabolized to three secondary metabolites namely; N,N-didesmethyltramadol (M3), N,N,O-tridesmethyltramadol (M4), and N,O-didesmethyltramadol (M5). In the phase II, the O-demethylated metabolites are excreted from urine by glucuronic acid and sulfate conjugation. In all species, M1, M1 conjugates, M2, M5 and M5 conjugates are the major metabolites, whereas M3, M4 and M4 conjugates are only formed in minor quantities (less than 1%) (4). Biliary excretion of Tramadol and its metabolites are negligible and from a quantitative point of view all metabolites as well

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as intact tramadol are almost completely excreted via the kidneys (5). In a study where a 50 mg oral dose of tramadol was given to 104 volunteers, mean 24-hrs urinary excretion for tramadol, M1 and M2 were 12%, 15% and 4% of the administered dose, respectively (6).

The mean total clearance of tramadol has been reported to be about 467 mL/min (approximately 28 L/hrs) and 710–742 mL/min (approximately 43–44 L/hrs) following intravenous and oral administration respectively with a mean elimination half-life of about 5-7 hrs (2).

Only one of tramadol's metabolites, namely O-desmethyltramadol (M1), is pharmacologically active. After oral administration of 100 mg of tramadol, T_{max} of M1 was about 1.4 hrs longer than tramadol with a C_{max} of no more than 18–26% of the parent drug (7).

Although the pharmacokinetic of tramadol and its active metabolite have been investigated extensively (7, 8), there are few studies on the non-stereoselective (9, 10) or stereoselective (11-13) pharmacokinetic properties of metabolites, and there is no published data on the pharmacokinetics of the metabolites in alternative biologic matrices, e.g. urine and saliva. Only in one study, the pharmacokinetic of tramadol in Plasma, urine and saliva has been investigated where it was shown that the C_{max} in saliva and urine occurred nearly at the same time as in plasma, and thereafter the plasma and saliva concentrations and the renal excretion rates decreased almost in parallel. Saliva and urinary concentrations were 7-8 and 43-46 folds higher than the corresponding plasma concentrations respectively (14).

In fact, saliva is the only fluid that has successfully been used as an alternative to plasma in several pharmacokinetic and pharmacotoxicologic studies (15, 16).

The purpose of this study was to investigate and compare in more details the pharmacokinetic of tramadol and its main three metabolites in most important biological samples including plasma, saliva and urine following administration of a 100 mg single oral dose to healthy volunteers and to find the relationships between saliva and plasma concentrations.

MATERIAL AND METHODS

Chemicals and Reagents

Pure tramadol, M1, M2, M5 and cis-tramadol as internal standard (IS) were kindly supplied by GrÜnenthal (Stolberg, Germany). HPLC-grade acetonitrile and methanol and analytical grade ethyl acetate and phosphoric acid (85%) were supplied by Merck (Darmstadt, Germany).

Participants and study design

Participants (12 male and 12 female healthy volunteers) who were with the mean age of 32 years (22-42 years), the mean weight of 72 kg (55-85 kg) and the mean height of 166 cm (151-182 cm) were informed about the purpose of the study and gave their consent to participate and received financial compensation. The protocol was approved by the Ethics Committee of Tehran University of Medical Sciences. Each participant underwent general physical examination, routine laboratory tests and urinary alyses.

Subjects were not allowed to take any other medication for 2 weeks before and throughout the study. Each subject fasted for 12 hrs before administration of 100 mg tramadol (two 50-mg Tradolan tablets) (Lannach, Austria) with 200 mL of water and continued to fast for the next 3 hrs. Standard breakfast and lunch were served 3 and 6 hrs after dosing, respectively. The subjects remained under close medical supervision up to 10 hrs after collection of the last blood samples.

Sample Collection

Blood samples (2 mL) were collected in heparinized glass tubes before (time 0) and 0.5, 1, 1.5, 2, 2.5, 3.5, 4.5, 6, 8, 10 and 24 hrs after drug administration. Plasma was harvested after separation from blood cells by centrifugation. Samples of mixed saliva - secreted by the different salivary glands (15) were obtained without any stimulation over a 2-min period immediately after each blood sampling time and then centrifuged. Plasma and saliva supernatants were separated and stored at -20 ° C until analysis.

After a pre-dose sample, urine was quantitatively collected in the following intervals: 0-1, 1-2, 2-3, 3-4, 4-5, 5-7, 7-9, 9-10, 10-14, 14-24 and 24-30 hrs. The volume of the urine pool was recorded, an aliquot of about 3 mL frozen at -20 ° was retained and the rest was discarded.

Analytical Method

Tramadol, M1, M2 and M5 in plasma, saliva or urine were determined by a previously described HPLC method (10). Briefly, all analytes were extracted with ethyl acetate and injected to a Knauer high-performance liquid chromatography (Berlin, Germany), equipped with a low-pressure gradient HPLC pump, a fluorescence detector, a Rheodyne injector with a 100 µL loop and an online degasser. Excitation and emission wavelength were 200 nm and 301 nm respectively. Separation was achieved by a Chromolith™ Performance RP-18e 100×4.6 mm column (Merck, Darmastadt, Germany) protected by a Chromolith™ guard cartridge RP-18e 5×4.6 mm. A methanol: water mixture (19:81, v/v) adjusted to pH of 2.5 by phosphoric acid at flow rate of 2 mL/min was used as mobile phase. Data acquisition was carried out by using ChromGate chromatography software (Knauer, Berlin, Germany). The calibration

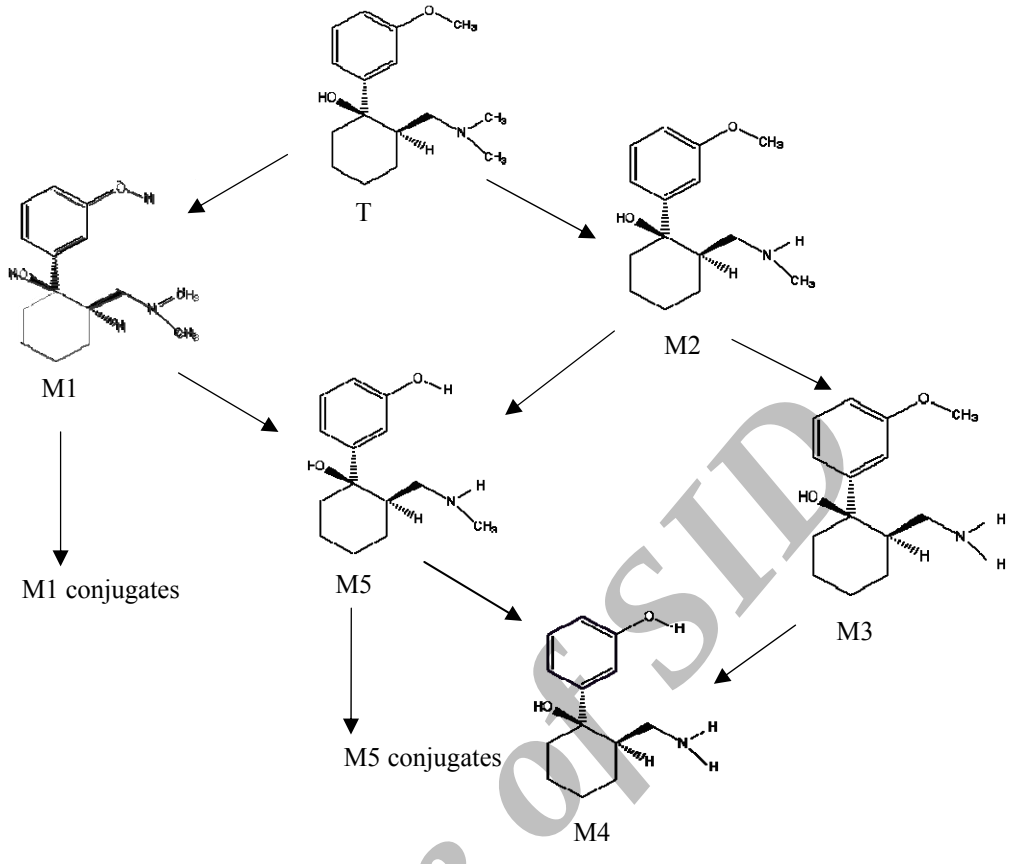

Figure 1. Metabolic pathway of tramadol.

curves were linear ($r^2 > 0.996$) in the concentration ranges in plasma, saliva and urine. The lower limit of quantification was 2.5 ng/mL for all compounds. Precision and accuracy studies showed an acceptable R.S.D. values (≤10.3%), (≤11.6%) and (≤13.6%) and accuracy of (86.9-111.9%), (93.6-108.7%) and (94.3-103.0%) in plasma, saliva and urine for both between- and within-day studies respectively. The lower limit of quantification was 2.5 ng/mL for all compounds. Mean recoveries of Tramadol, M1, M2 and M5 from above biological fluid samples were ≥86.2%, ≥76.9%, ≥80.1% and ≥90.5% respectively.

Pharmacokinetic Calculation

The pharmacokinetics of tramadol and its metabolites were determined by non-compartmental analysis. Maximum plasma concentrations (C_{max}) and their corresponding times (T_{max}) were recorded as observed. Elimination rate constant (λ) was estimated as the absolute value of the slope of least-square linear regression of the terminal phase of the logarithmic plasma concentration– time curve. The plasma terminal half-life ($t_{1/2}$) was calculated as 0.693/λ. Area under the plasma concentration–time curves from time zero to the time of last quantifiable concentration (AUC_{0-t}) was calculated using the linear trapezoidal method. Area under the plasma concentration–time curves from time zero to the infinite time ($AUC_{0-\infty}$) was calculated as the sum of corresponding AUC_{0-t} and Ct/λ values. Plasma oral clearance (CL/F) was calculated as Dose/$AUC_{0-\infty}$. Apparent volume of distribution (V_d/F) was determined using the equation $V_d/F = (Dose/AUC_{0-\infty})/\lambda$. Renal clearance (CLr) was determined using the equation $CLr = A/AUC_{0-t}$ where A is the amount excreted into the urine from time zero to the time of the last quantifiable concentration. Cumulative renal excretion of unchanged tramadol and its metabolites extrapolated to infinity ($A_{e\infty}$) was calculated by adding the amount determined experimentally after 30 hrs (Ae_{0-30}) to the residual amount $Ae_{30-\infty}$ which was computed from the renal excretion rate of the last sampling interval (Ae_{24-30} /6) and the elimination rate constant (λ) according to the equation $Ae_{30-\infty} = (A_{24-30}/6) \times e^{-\lambda} \times 1.5 / \lambda$.

Statistical analyses

Statistical evaluation of the concentration data and of the individual pharmacokinetic parameters was performed descriptively by calculating means with standard deviations (SD) and coefficient of variations (CV (%)). Correlations between different variables were analyzed by regression analysis.

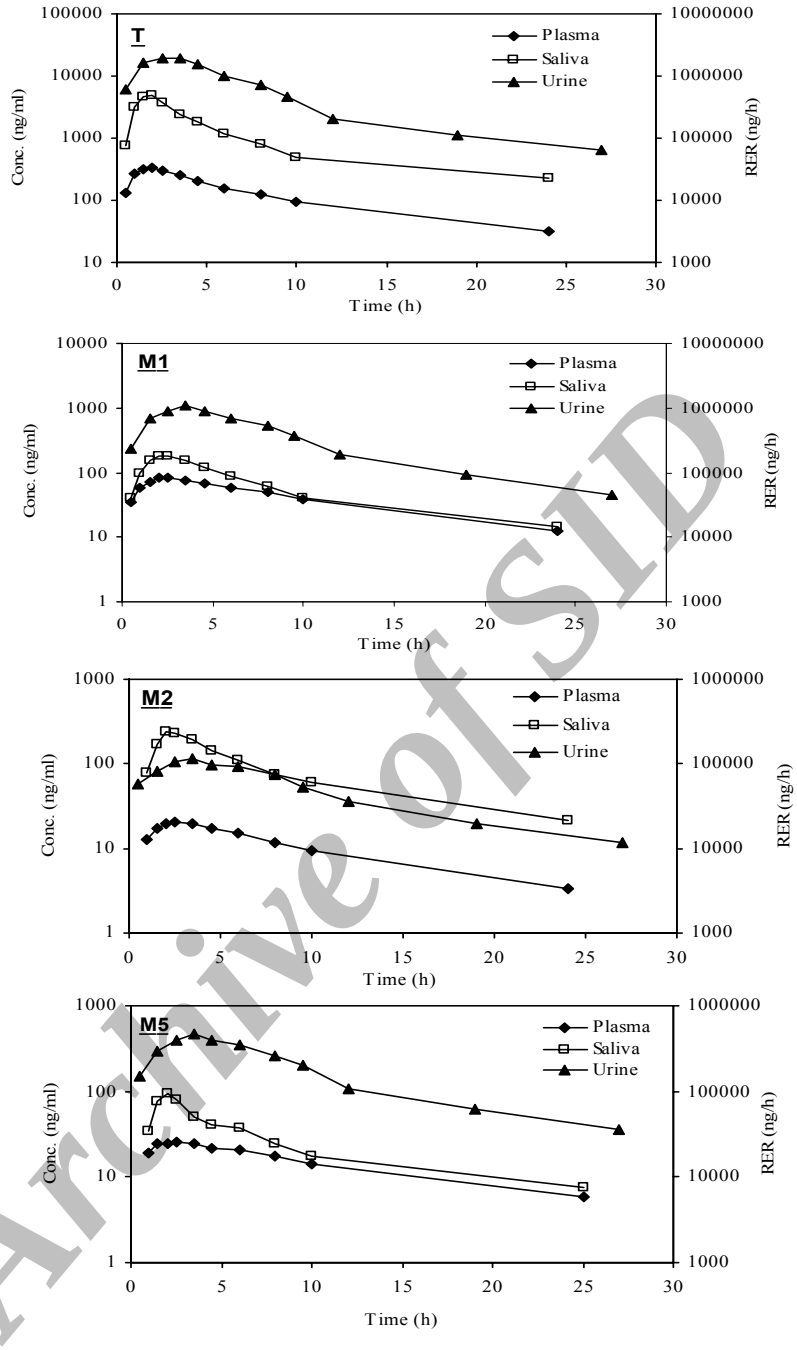

Figure 2. Mean concentration–time profile in plasma and saliva and renal excretion rate of T, M1, M2 and M5 after administration of 100 mg single oral dose of Tramadol.

RESULTS

Concentration–time profiles and Pharmacokinetics calculations

Tramadol

Simultaneous collection of blood, saliva and urine samples allowed to compare the time-concentration profiles of tramadol in relatively all main specimens. Respective time courses of tramadol in the healthy volunteers are depicted in figure 2 and the corresponding pharmacokinetic variables are summarized in table 1.

As it is shown in figure 2, the concentration-time courses of tramadol in saliva and plasma followed similar but not identical profiles. After 0.5 hrs of administration, tramadol concentration in saliva considerably exceeded the plasma concentration. However, saliva maximum concentration occurred nearly at the same time as in plasma. Thereafter, plasma and saliva concentration and renal excretion

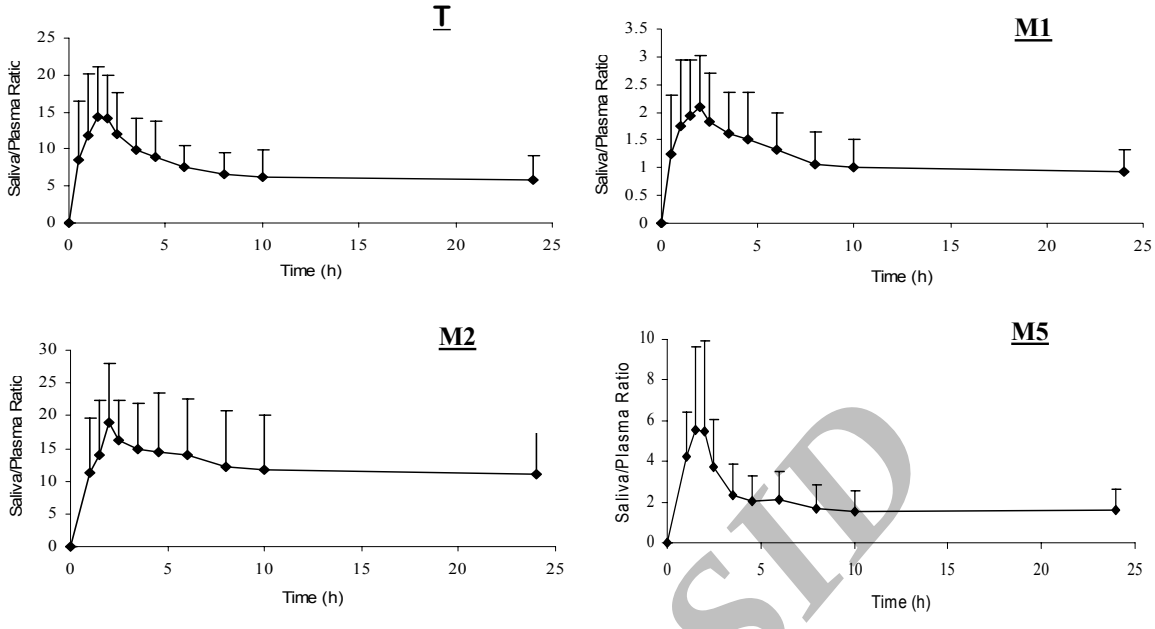

Figure 3. Saliva/Plasma ratio -time profile of T, M1, M2 and M5.

rate decreased almost in parallel.

Time-course profile for the Saliva/Plasma ratio during 24 hrs after drug administration is presented in figure 3. The ratio exhibited a maximum value of 14.4 ± 6.7 at 1.5 hrs, corresponding to the plasma T_{max} of tramadol. In the post-absorption phase, the ratio decreased gradually to a mean value of 6.5±0.4 at 6 hrs after drug administration and remained almost constant thereafter, showing a parallel decrease in saliva and plasma concentration profiles in post absorption parts.

Regardless of the variation of Saliva/Plasma ratio during the time course of tramadol administration, mean tramadol plasma concentrations showed a significant correlation with respective salivary concentrations (r^2 = 0.918). As shown in figure 4, separation of the corresponding time points to pre- and post absorption components, resulted in an enhanced correlation coefficient due to the profile of Saliva/Plasma ratio which has a non-linear fraction in pre absorption part and a linear section in the other part. On the average, saliva concentrations were higher than the corresponding plasma concentrations by a factor of 5.9±3.3 to 14.4±6.7. The $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of saliva concentration-time profile were higher than the corresponding values in plasma profiles respectively by a factor of 9.0±4.1 and 8.8±4.8. Elimination half-life of tramadol was 7.2 ± 0.8, 7.8 ± 1.5 and 6.6 ± 0.8 hrs in plasma, saliva and urine respectively. Cumulative renal excretion of tramadol ($A_{e\infty}$) was 17.5±6.5 % of the administered oral dose. Apparent plasma clearance (CL/F) and renal clearance (CLr) were 595 ± 130 and 115 ± 45 mL/min respectively.

O-desmethyltramadol (M1)

Plasma and saliva concentration and renal excretion rate profiles of M1 are presented in figure 2, and their related pharmacokinetic parameters are listed in table 2.

The profiles of saliva and urine exceeded the plasma profiles significantly at first and then decreased almost in parallel. T_{max} of M1 of all three profiles occurred nearly 1 hrs after T_{max} of tramadol.

Saliva/Plasma ratio of this metabolite was 2.1±0.9 at 2 hrs after administration (Figure 3). Like the parent drug, the ratio increased initially and decreased steadily to a constant value of about 1.0±0.07 at the last three time points. Mean M1 plasma concentration at any time point showed a strong correlation with respective salivary concentrations (r^2 = 0.970). However, unlike tramadol no increase in r^2 was observed when the line was dissected to pre and post absorption phases (Figure 4).

On the average, saliva maximum concentrations were higher than the corresponding plasma concentrations by a factor of 2.1 ± 0.9 to 0.9 ± 0.4. $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of saliva-time concentration were higher by a factor of 1.6 ± 0.8 and 1.6 ± 0.7 in comparison to the corresponding values in plasma profiles, respectively. The terminal half-life of M1 was 7.7± 1.4, 7.8 ± 2.3 and 6.8 ± 1.1 hrs based on plasma, saliva and urinary profiles respectively. Cumulative renal excretion of M1 ($A_{e\infty}$) was 13.1±4.9 % of the dose and the CLr was 193.9 ± 67.6 mL/min after oral administration.

N-desmethyltramadol (M2)

Mean plasma and saliva concentration–time and

Table 1. Pharmacokinetic parameters of **Tramadol** in healthy subjects after a single oral dose of 100-mg tramadol tablets (n=24).

Parameters	Tramadol		
	Plasma	**Saliva**	**Urine**
C_{max} (ng/mL)	349.3 ± 76.7	5702.3 ± 2308.4	
T_{max} (hrs)	1.6 ± 0.4	2.0 ± 0.4	
$AUC_{(0-t)}$ (ng.hrs/mL)	2748.2 ± 768.5	23371.5 ± 8794.6	7861.2 ± 2034.7*
$AUC_{(0-\infty)}$ (ng.hrs/mL)	3159.0 ± 1082.0	27075.4 ± 10404.2	
$T_{½}$ (hrs)	7.2 ± 0.8	7.8 ± 1.5	6.6 ± 0.8
CL/F (mL/min)	594.7 ± 129.5		
V_d/F (L)	375.3 ± 77.3		
CLr (mL/min)			114.7 ± 44.5
A_{0-30} (mg)			16.2 ± 6.2
$A_{e\infty}$ (mg)			17.5 ± 6.5

N = 24, mean ± SD.
*µg.hrs

Table 2. Pharmacokinetic parameters of **M1** in healthy subjects after a single oral dose of 100-mg tramadol tablets (n=24).

Parameters	O-desmethyltramadol (M1)		
	Plasma	**Saliva**	**Urine**
C_{max} (ng/mL)	88.7 ± 30.3	245.3 ± 156.6	
T_{max} (hrs)	2.4 ± 0.7	2.3 ± 0.7	
$AUC_{(0-t)}$ (ng.hrs/mL)	929.3 ± 256.1	1342.9 ± 884.5	
$AUC_{(0-\infty)}$ (ng.hrs/mL)	1094.2 ± 311.9	1579.4± 985.8	
$T_{½}$ (hrs)	7.7 ± 1.4	7.8 ± 2.3	6.8 ± 1.1
CLr (mL/min)			193.9 ± 67.6
A_{0-30} (mg)			11.2 ± 3.8
$A_{e\infty}$ (mg)			13.1 ± 4.9

N=24, mean ± SD.

renal excretion rate curves of N-desmethyltramadol (M2) are depicted in figure 2 and corresponding pharmacokinetic variables are summarized in table 3.

Profiles of saliva and urine significantly exceeded the plasma profile and decreased almost in parallel. The T_{max} of M2 in saliva and urine compared to plasma occurred approximately 0.5 hrs later in saliva and urine in comparison to plasma. Maximum saliva concentration was higher than those of plasma with a mean value of 15.1 ± 6.1. Plasma $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ were higher than their corresponding plasma parameters.

Figure 3 represents the saliva/plasma ratio time course of M2 metabolite with a maximum value of 19.0 ± 9.0 at 2 hrs after tramadol administration. As shown in figure 4, a significant correlation was observed between mean saliva and corresponding plasma concentration especially after dissecting the profile to pre- and post absorption parts.

The terminal half-life of M2 was 10.3 ± 2.1, 11.0± 3.5 and 9.5 ± 3.5 hrs according to plasma, saliva and urine profiles, respectively. Cumulative renal excretion of M2 ($A_{e\infty}$) was 1.5 ± 0.7 % of the dose and the CLr was 116.1 ± 61.8 mL/min after oral administration.

O,N-didesmethyltramadol (M5)

Plasma and saliva concentration and renal excretion rate profiles of M5 are presented in figure 2 and the corresponding pharmacokinetic parameters are shown in table 4.

Like other analytes, M5 concentration in saliva and urine noticeably exceeded the plasma concentration with the maximum concentration occurring nearly 0.5 hrs after T_{max} of the plasma, then all profiles decreased almost in parallel.

The saliva/plasma ratio time course profile is presented in figure 3. Enhanced correlation was observed by separation of pre- and post absorption segments.

C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of M5 in saliva were 5.6 ± 3.6, 2.8 ± 1.6 and 2.6 ± 1.5 folds higher than the corresponding values in plasma, respectively.

Table 3. Pharmacokinetic parameters of **M2** in healthy subjects after a single oral dose of 100-mg tramadol tablets.

Parameters	N-desmethyltramadol (M2)		
	Plasma	**Saliva**	**Urine**
C_{max} (ng/mL)	23.1 ± 11.4	252.7 ± 183.2	
T_{max} (hrs)	2.8 ± 1.0	3.1 ± 0.7	
$AUC_{(0-t)}$ (ng.hrs/mL)	231.3 ± 131.1	1880.2 ± 1565.5	
$AUC_{(0-\infty)}$ (ng.hrs/ mL)	288.7 ± 165.6	2354.8 ± 2180.9	
$T_{½}$ (hrs)	10.3 ± 2.1	11.0 ± 3.5	9.5 ± 3.5
CLr (mL/min)			116.1 ± 61.8
A_{0-30} (mg)			1.1 ± 0.6
$A_{e\infty}$ (mg)			1.5 ± 0.7

N=24, mean ± SD.

Table 4. Pharmacokinetic parameters of **M5** in healthy subjects after a single oral dose of 100 -mg tramadol tablets.

Parameters	O,N-didesmethyltramadol (M5)		
	Plasma	**Saliva**	**Urine**
C_{max} (ng/mL)	30.0 ± 11.7	106.2 ± 80.7	
T_{max} (hrs)	2.7 ± 1.4	3.2 ± 0.8	
$AUC_{(0-t)}$ (ng.hrs/mL)	324.2 ± 140.8	651.4 ± 481.1	
$AUC_{(0-\infty)}$ (ng.hrs/mL)	445.2 ± 231.8	772.5 ± 526.0	
$T_{½}$ (hsr)	10.2 ± 4.0	11.0 ± 2.5	12.5 ± 3.1
CLr (mL/min)			252.0 ± 91.5
A_{0-30} (mg)			5.0 ± 2.1
$A_{e\infty}$ (mg)			6.6 ± 2.7

N=24, mean ± SD.

Elimination half-life of M5 were 10.2 ± 4.0, 11.0 ± 2.5 and 12.5 ± 3.1 hrs in plasma, saliva and urine profiles respectively. Cumulative renal excretion of M5 ($A_{e\infty}$) was 6.6 ± 2.7% of the dose after oral administration and its CLr was 252.0 ± 91.5 mL/min.

DISCUSSION

To the best of our knowledge, this investigation is the first published study to provide fundamental data on the basic pharmacokinetics of tramadol as well as its main metabolites in plasma, saliva and urine. All pharmacokinetic parameters of tramadol in plasma analysis, were in agreement with results of the previous studies on the parent compound.

In accordance with the published data, about 16% of tramadol was found in collected urine samples (3, 6, 13). This variation in urinary excretion may results from ethnic difference

There are few data on urinary excretion of tramadol and all three main metabolites. While Rudaz et al. reported almost the same excretion amount for M1 and M5 in urine, (i.e. 16 and 15% of the oral dose respectively) the excretion proportion of M1 and M5 in this study were found to be 11% and 5% of the oral dose respectively. Only small amount of M2 metabolite was observed in the present study (approximately 1.1% of the oral dose), while the excreted amount of this metabolite was found between 2-4 percent in previous reports (3, 6, 13).

Renal clearance of tramadol and M1 were 114.7 ± 44.5 and 193.9 ± 67.6 mL/min respectively, which were in good agreement with 110 and 188 mL/min for tramadol and M1 reported by Liao S et al. (8). Renal clearance of M2 and M5 reported for the first time in this study are 116.1 and 252.0 mL/min respectively.

As mentioned above, tramadol appeared in saliva in concentrations remarkably higher than those of plasma (Figure 2). By using to the Henderson-Hasselbalch equation and considering the lowest recorded pH in volunteers, the maximum theoretical saliva/plasma ratio for tramadol was calculated to be around 4.2˚ (15). Nevertheless, mean observed saliva/plasma ratio was 14.4 at peak tramadol concentrations and 6.1 at 24 hrs after drug administration. The difference between calculated and observed saliva/plasma ratio may be attributed to several factors. Drugs are generally incorporated into saliva by passive diffusion because of a concentration gradient in which only the free fraction of the drug (not bounded to proteins) diffuses through lipid membranes from plasma to saliva. It

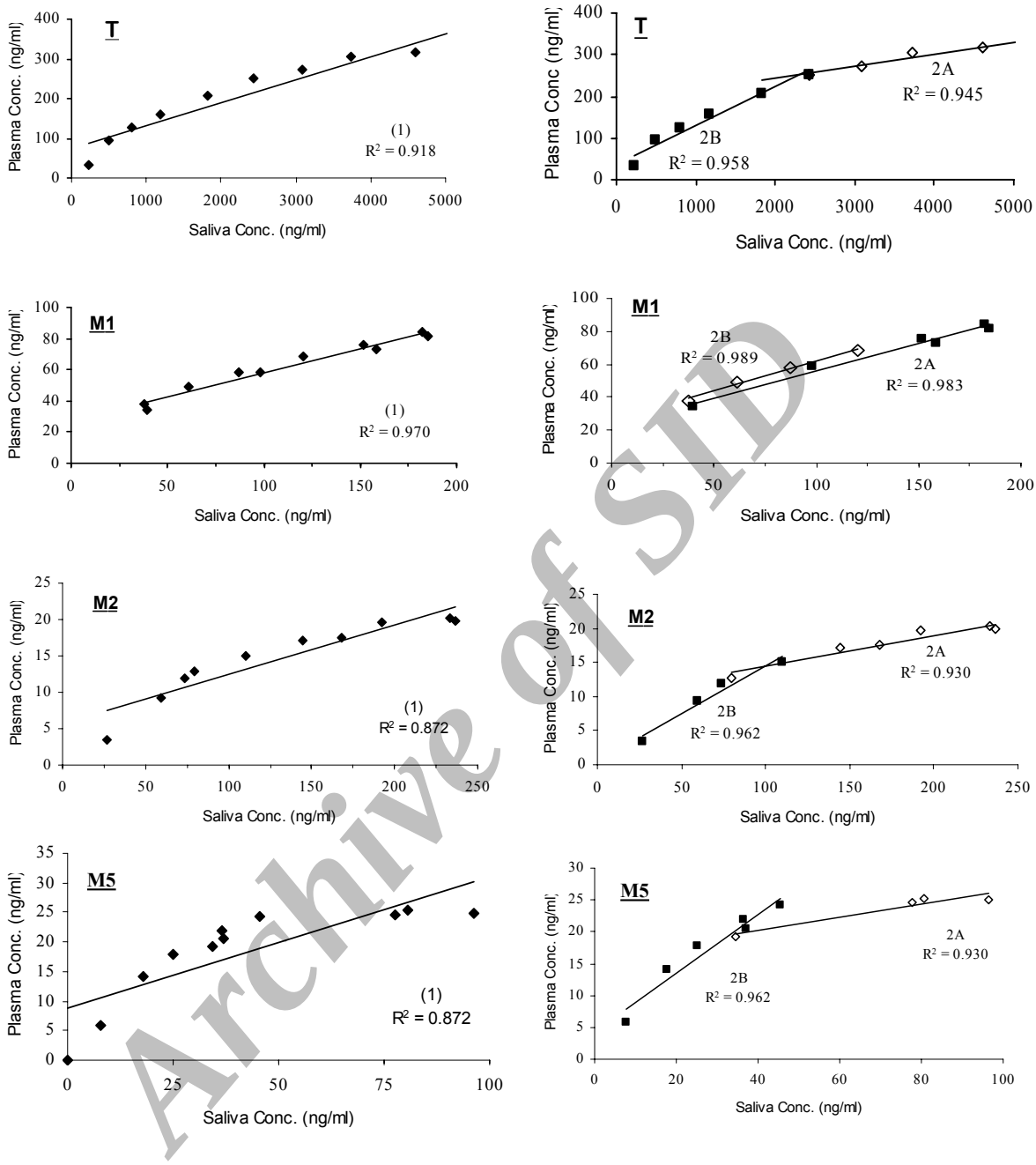

Figure 4. Correlation between mean plasma and saliva concentrations, before (1) and after separating into two components of pre- (2A) and post-absorption (2B).

should be noted that saliva has little protein binding capacity in comparison to plasma. On the other hand such low plasma protein binding of tramadol (about 20 %) resulted in relatively high diffusion (concentration gradient) from plasma into the saliva. In addition, passage across the cell membranes is favored for low-molecular weight molecules, such as tramadol (15). Furthermore, tramadol is a basic drug with a pKa value of 8.1 and because of more acidic pH of saliva (pH~6.7) in the absence of salivary flow stimulation, tramadol is converted to its ionized form and consequently accumulates in saliva. Stimulation of saliva secretion increases the pH to values approaching plasma pH and in the case of such a basic drug, this reduces the salivary drug concentration, and also the variability in saliva/plasma ratios is narrowed (15).

The fact that saliva was collected without flow stimulation may partly explain the over estimated tramadol saliva/plasma ratios. The serotonergic

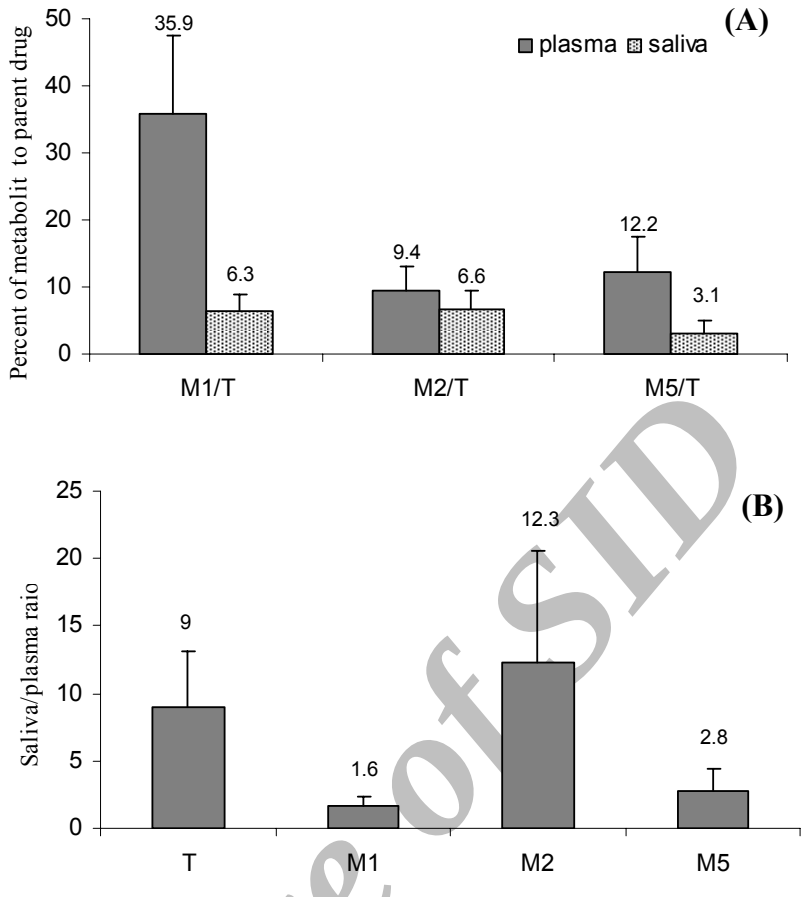

Figure 5. Percent of metabolite/tramadol in saliva and plasma (A), and saliva/plasma $AUC_{(0-t)}$ ratio for all compounds (B).

effect of tramadol results in lower saliva production which in turn concentrates this fluid. This could partly explain the above-mentioned observation. In fact, individuals receiving tramadol usually exhibit dry mouth which is in the support of the above reasoning (1). Also, tramadol most probably impairs salivary flow through its sympathomimetic effects, producing a sympathetic constriction of the salivary bed (1). Consequently, buffering capacity, which is maximal under conditions of flow stimulation, can be reduced, and the pH of mixed saliva obtained from the oral cavity (present study) may not be the same as the pH at the site of saliva secretion (15). Hence, a dynamic concentration gradient takes place, which probably produces tramadol saliva/plasma ratios higher than those calculated by the Henderson–Hasselbalch equation. On the other hand, Lintz et al. reported 7 to 8 folds higher salivary than corresponding plasma tramadol concentration after collection of saliva by glass beads stimulation for five minutes (14). This observation may reduces the importance of saliva collection method to explain the high saliva/plasma ratio for tramadol. Active transport may also explain high saliva/plasma ratio of tramadol and its high variability between individuals, no report was found to confirm active transport of tramadol into saliva.

As it was shown in figure 5, all metabolites were excreted considerably into saliva. Although M2 represented the lowest percent among all detected metabolites in plasma, this metabolite showed the highest saliva/plasma ratio among all excreted compounds and the same metabolite/tramadol ratio was observed for M1 and M2 regardless of much higher concentration of M1 in plasma ($AUC_{(0-t)}$ was compared) (Figure 5 panel A).

Higher saliva/plasma ratio observed for M2 in spite of its low plasma concentration could be best explained by its polarity. Due to inter-molecular hydrogen binding that could increase after N-demethylation of tramadol to form M2 metabolite, decrease in the polarity of M2 compared to the parent compound is expected. Decreased molecular polarity may in turn results in easier permeation into saliva. M5 also showed a higher saliva/plasma ratio than M1 (2.8 versus 1.6) which could also be due to lower polarity of M5 compared to M1. This difference in polarity was also confirmed by the order of retention times of tramadol and its metabolites in the reversed phase chromatography which were 2.1, 2.5, 4.9 and 6.4 min for M1, M5, tramadol and M2 respectively (10).

Higher concentrations of tramadol and its

metabolites in saliva compared to their theoretical values (according to Henderson–Hasselbalch equation) could be explained by the presence of an active transport system with different affinities for these compounds. This is in agreement with a report on active transport of the parent compound by P-glycoproteins (17). Further investigations are required to check this hypothesis for tramadol metabolites.

Regardless of difference in saliva/plasma ratios, relatively good correlations were achieved between mean saliva and respective plasma concentrations for all compounds during the sampling period. As shown in figure 4, dissection of time points to pre- ($t \leq t_{max}$) and post-absorption ($t > t_{max}$) components, resulted in an enhanced correlation in each section.

Higher saliva/plasma ratios in the absorptive phase are in accordance with a rational explanation based on an anatomical-physiological hypothesis. During the absorption phase, drug concentration in arterial blood is higher than in peripheral venous blood and after absorption is ceased, concentrations are practically equal. Because the salivary glands are well perfused, the equilibrium between the membrane permeable drug fraction in arterial blood and drug in saliva is rapidly established. This is reflected in an elevated saliva/plasma ratio during the absorption phase (18). Taking these plasma-saliva correlations into account, the measurement of tramadol in saliva appears to be a suitable alternative to plasma analysis in clinical and toxicological situations where detection of recent abuse is requested. Despite changes in the saliva/plasma ratio during the time course for tramadol in saliva and plasma, correlation between tramadol concentrations in two biological fluids indicates that salivary concentrations of this drug may be a predictor of plasma concentrations. Because of the higher concentrations encountered, saliva exhibits a larger time window for detection of tramadol administration with a much less invasive method than with plasma and without specific requirements for sample collection, thus facilitating on-site sample collection and drug testing.

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