

Bardia Jamali, Behjat Sheikholeslami, Yalda Hosseinzadeh Ardakani, Hoda Lavasani and Mohammad-Reza Rouini*

Evaluation of the Ecstasy influence on tramadol and its main metabolite plasma concentration in rats

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Abstract

Background: Tramadol is prone to be abused alone, or in combination with 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy). It was reported that 95% of people with a history of substance abuse in the United States used tramadol in 2004. According to the WHO report in 2016, there was a growing number of tramadol abusers alone or in combination with psychoactive substances such as MDMA in particular in some Middle East countries. Higher concentrations of tramadol in plasma may lead to adverse drug reactions or lethal intoxication. In this study, the effect of MDMA on the pharmacokinetics of tramadol was examined in male rats.

Methods: The effect of MDMA on T_{max} , C_{max} , area under the curve, elimination rate, and half-life of tramadol and its metabolites was examined. Two control and two treatment groups were designed. The treatment groups received MDMA 18 h before the administration of tramadol. Jugular vein blood samples were analyzed by high-performance liquid chromatography with fluorescent detector to determine the concentrations of tramadol and its metabolites. Independent-sample t-test was used to define the differences between pharmacokinetic parameters of control and treatment groups.

Results: When tramadol administered intraperitoneally, the absorption rate of this drug was reduced, and a lower C_{max} (40%) with longer T_{max} (eight-fold) was achieved.

MDMA exerted greater inhibitory effects on cytochrome P450 3A4 (CYP3A4) than on cytochrome P450 2D6 (CYP2D6). The M_2 metabolite ratio was reduced by half, and because of the inhibition of M_2 production, the M_1 plasma concentration slightly increased.

Conclusions: According to the obtained data, MDMA treatment affected the absorption, distribution and metabolism phases of tramadol. This treatment increased the concentration of tramadol if administered intravenously and can latent the absorption of tramadol in oral route. However, MDMA was introduced as CYP2D6 inhibitor; in this study, MDMA inhibited CYP3A4 isoenzymes as well. This finding is important for the compounds that are metabolized through CYP3A4. It can be proposed that in abusers of MDMA who only receive tramadol for medical or nonmedical purposes in short intervals, the dangers of the intravenous administration of tramadol should be considered, and if tramadol is administered orally, the desired effect may not be achieved at the routine dose.

Keywords: CYP3A4; drug-drug interaction; Ecstasy; MDMA abusers; pharmacokinetic; tramadol.

Introduction

Drug-drug interaction is one of the most problematic issues in patient management. As a drug class, opioids such as codeine, tramadol and morphine attract more attention to this issue because of their inherent life-threatening toxicity and intersubject variability [1]. The number of users of this class of drugs has quadrupled over the past two decades [2]. Among opioids, tramadol is a widely used synthetic centrally acting compound, with a similar structure to codeine [3, 4], but unlike other opioids, it is not a restricted drug in most of the countries where it is marketed. The nonmedical uses of this compound have increased 2.3 times from 2003 to 2010 among people older than 12 years [2, 5].

This compound is prone to be abused because of its psychotropic effects and its ability to reduce withdrawal

*Corresponding author: **Mohammad-Reza Rouini**, Faculty of Pharmacy, Department of Pharmaceutics, Biopharmaceutics and Pharmacokinetics Division, Tehran University of Medical Sciences, Tehran 14155-6451, Iran, E-mail: rouini@tums.ac.ir

Bardia Jamali, Yalda Hosseinzadeh Ardakani and Hoda Lavasani: Faculty of Pharmacy, Department of Pharmaceutics, Biopharmaceutics and Pharmacokinetics Division, Tehran University of Medical Sciences, Tehran, Iran

Behjat Sheikholeslami: Faculty of Pharmacy, Lorestan University of Medical Sciences, Khorramabad, Iran

symptoms. It was reported that 95% of people with a history of substance abuse in the United States used tramadol in 2004. According to the WHO report in 2016, there was a growing number of tramadol abusers alone or in combination with psychoactive substances such as 3,4-methylenedioxymethamphetamine (MDMA) in particular in some Middle East countries. The rate of tramadol abuse among Chinese drug abusers was 13.3 in 2009 [6].

Tramadol is a mild agonist for μ -opioid receptors, inhibiting the reuptake of noradrenaline and serotonin in descending inhibitory pathways and increasing the efflux of serotonin in the central nervous system [3, 7–9]. Because of this unique mechanism of action, tramadol has a broad spectrum of applications. This compound is frequently prescribed for the management of moderate to severe pain in cancerous or noncancerous patients [3], as

well as psychiatric disorders such as depression, anxiety and Tourette's syndrome [4, 9].

Tramadol is mainly metabolized via two members of the CYP450 isoenzyme family, namely, cytochrome P450 2D6 (CYP2D6) and cytochrome P450 3A4 (CYP3A4). The former produces *O*-desmethyltramadol (M_1), and the latter produces *N*-desmethyltramadol (M_2). Although it is minor, CYP2B6 also contributes to M_2 production [1, 3]. M_1 has 300 times greater affinity for binding to μ -opioid receptors than tramadol, whereas M_2 is known as an inactive metabolite of the drug. Both M_1 and M_2 can be further metabolized to form *N*-*O* didesmethyltramadol (M_5), which is recognized as a partial agonist for μ -opioid receptors (Figure 1) [3, 11] and has demonstrated no *in vivo* activity [12].

Among these metabolism pathways of tramadol, *O*-demethylation, which is mainly mediated by the

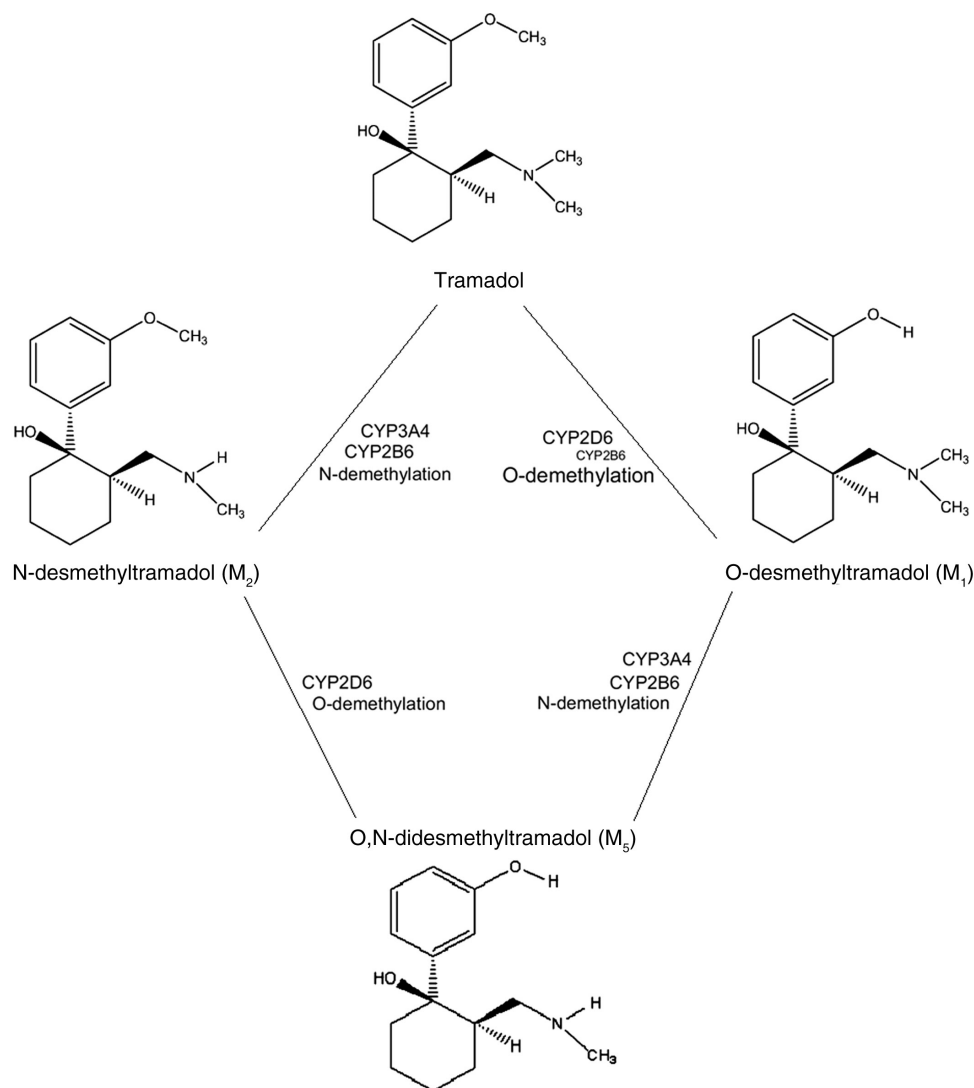


Figure 1: The proposed metabolism pathways for tramadol [7, 10].

CYP2D6 isoenzyme, is the pathway of most concern. The CYP2D6 isoenzyme forms 2%–5% of the CYP450 family and exerts saturable nonlinear kinetics [7]. This isoenzyme plays a role in the metabolism of 25% of drugs such as opioids, antidepressants, and neuroleptic medications [7]. It is obvious that the induction or inhibition of CYP2D6 can change the pattern of tramadol metabolism.

Because of tramadol's effects on the serotonergic system and opioid receptors, it is prone to be abused alone or in combination with amphetamine-like compounds, such as 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy). Recently, some restrictions on the availability of this drug have been proposed [2]. Amphetamine-like compounds such as MDMA are one of the most commonly abused drug in the world [13], and their consumption has increased among youth in Iran [14, 15]. Recently, it was reported by Khajeamiri et al. [16] that tramadol was one of the active ingredients in the Ecstasy-containing pills seized in Iran. Moreover, tramadol was recently found at high concentration levels in street heroin seizures in the Middle East region [17]. MDMA, which is used as a pill in night clubs or rave parties, is a releaser and presynaptic reuptake inhibitor of serotonin [18]. This compound is metabolized extensively by CYP2D6 to give the main neurotoxic metabolite [18, 19]. It has been established that MDMA is a well-known irreversible inhibitor of CYP2D6 [20, 21].

Thus, we designed a study to examine the effect of MDMA pretreatment on the metabolism of tramadol when administered either intravenously (IV) or intraperitoneally (IP) in an animal model. The finding of this study may be useful for managing the patient with a history of MDMA abuse or explain the reported adverse effects in this population.

Materials and methods

Materials

Tramadol, M_1 , M_2 and M_3 , along with *cis*-tramadol (used as an internal standard), were generously supplied by Grünenthal (Stolberg, Germany). MDMA powder was purchased from Lipomed Pharmaceutical (Switzerland). All of these compounds were used as racemic mixtures. Methanol of high-performance liquid chromatography (HPLC) grade and all other chemicals were of analytical grade and were purchased from Merck (Darmstadt, Germany). These chemicals were used without further purification. Xylazine (2%; Alfasan, Woerden, the Netherlands), ketamine (10%; Alfasan, Woerden, the Netherlands), an injectable solution of normal saline (Darupakhsh, Tehran, Iran), and heparin sodium (10,000 IU; Caspian Tamin, Tehran, Iran) were acquired from a local supplier. Ultrapure water was obtained from Millipore Direct-Q System (Molsheim, France).

Animal studies

The study protocol was approved by the Animal Ethical Committee of Tehran University of Medical Sciences (89/D/230/766, 13 June 2010).

Male Sprague-Dawley rats weighing between 250 and 300 g were used. These animals were housed in standard cages at a controlled temperature and humidity with a 12-h light/12-h dark cycle in the animal care room of the Faculty of Pharmacy, Tehran University of Medical Sciences. They had free access to water and standard rat chow. In this study, Sprague-Dawley rat was chosen as an animal model because of the high sequential homology between CYP2D, CYP2B and CYP3A in rat and humans [22, 23].

Animals were anesthetized by IP injection of a ketamine/xylazine mixture (ketamine at 100 mg/kg and xylazine at 10 mg/kg). The polyethylene-silicone rubber cannulas were implanted in their right jugular veins according to a reported surgical operation [24] and filled with a heparinized normal saline solution to prevent clotting. The cannulas were tunneled under their skin and externalized on the dorsal surface of the neck. The animals were left overnight to achieve complete recovery.

The rats were separated randomly into four groups ($n=6$ rats/group). Using a 1-mg/mL solution of MDMA and a 10-mg/mL solution of tramadol in normal saline, the treatment groups (two groups of six rats) received MDMA (0.5 mg/kg) via IV cannula 18 h before the administration of tramadol, whereas animals in the control groups (two groups of six rats) received normal saline instead of MDMA. Both control and treatment groups received tramadol (10 mg/kg) via the IP or the IV route 18 h after pretreatment with either MDMA or vehicle. Noticeably, the animals remained unrestrained during the entire drug administration and sampling periods.

Notably, according to our preliminary study and unpublished data in male Sprague-Dawley rats, IV administration of MDMA (0.5 mg/kg) produced an initial plasma concentration of 204 ± 29 ng/mL, which was similar to reported plasma concentrations of MDMA (106–465 ng/mL) in people who ingested MDMA pills [25]. Moreover, 18 h after the IV administration of MDMA (0.5 mg/kg) in male Sprague-Dawley rats, the concentration of MDMA and its metabolites was lower than the limit of quantification of our assay method for MDMA (2 ng/mL). Thus, the chance of MDMA interfering in the analysis of tramadol and its metabolites was unlikely.

Blood samples (0.3 mL) were collected at appropriate time intervals (0–300 min) via flow through the cannula into preheparinized polypropylene microtubes. The samples were centrifuged at 1000 g for 10 min, and the plasma samples were kept frozen at -20°C until drug analysis.

Analytical methods

Instruments: The chromatographic system consisted of a double-reciprocating HPLC pump (Knauer, model K-1001, Darmstadt, Germany) equipped with an online degasser, a fluorescence detector (Knauer, model RF-10AXL, Darmstadt, Germany), and a Knauer injector with a 100- μL loop. Instrument control, data acquisition, and processing were performed by means of EZChrom Elite software (Knauer, version 3.1.7, Darmstadt, Germany).

Tramadol assay: A previously reported HPLC method with liquid-liquid extraction and fluorescent (FL) detection by Ardakani and

Rouini [10] was used for the determination of the concentrations of tramadol and its metabolites. The method was validated according to the FDA guidelines for bioanalytical method validation [26].

Briefly, 120 μL of plasma was extracted with ethyl acetate. After the evaporation of the organic phase, the residue was reconstituted in 120 μL of mobile phase. A 100- μL sample of the obtained aliquot was injected into the HPLC/FL.

The FL detector wavelength was fixed at 200 nm for excitation (λ_{ex}) and at 301 nm for emission (λ_{em}). The mobile phase was a mixture of methanol and water (19:81 v/v). The pH level of the water was adjusted to 2.5 by phosphoric acid. The separation of the analytes was performed at ambient temperature (25 °C) in isocratic mode, with a flow rate of 2 mL/min on the Chromolith® Performance RP-18e (100 mm \times 4.6 mm) column, which was protected by a Chromolith® RP-18e Guard Cartridge (5 mm \times 4.6 mm) from Merck.

Pharmacokinetic calculation

For pharmacokinetic parameter calculation, after administration of tramadol, the compartmental model and the Monolix 4.1 software (Lixoft SAS, France) were used. The best model was chosen according to Akaike information criterion. The highest observed plasma concentration (C_{max}) and time required to reach C_{max} (T_{max}) were determined via experimental data. The area under the concentration-time curve was calculated by trapezoidal method.

Statistical analysis

The data are presented as the mean \pm standard error of the mean (SEM). Dixon *Q* test was used for the identification of data outliers. The metabolite ratio was defined as metabolite concentration divided by tramadol concentration at the same time. Student's independent-sample *t*-test ($p < 0.05$) was used to determine differences between pharmacokinetic parameters in treatment and control groups. The Microsoft Office Excel® software 2007 was used to constructing the diagrams.

Results

Calibration curves were constructed for tramadol, M_1 , M_2 and M_5 in plasma (25–800 ng/mL) by linear regression, without weighting. All calibration curves showed reasonable linearity in the selected ranges ($r > 0.99$). The differences between the slopes and the zero were significant ($p < 0.05$), whereas the intercepts showed no significant differences with zero ($p > 0.05$). The inaccuracy and the precision (relative standard deviation) of the method were $< 15\%$ and $< 10\%$, respectively. The most informative calibration and validation parameters of the assay method are presented in Table 1.

Tramadol IV administration

When tramadol was administered via the IV route, the best model according to Akaike criterion, which was used

Table 1: Calibration and validation parameters for tramadol, M_1 , M_2 and M_5 in male Sprague-Dawley rat plasma.

	Tramadol	M_1	M_2	M_5
Calibration range, ng/mL	25–800	25–800	25–800	25–800
Calibration points	5	5	5	5
Correlation coefficient (<i>r</i>)	0.995	0.990	0.994	0.990
Slope	0.002	0.001	0.002	0.001
Intercept	0.016	0.003	−0.002	0.008
SE of slope	<0.001	<0.001	<0.001	<0.001
SE of calibration curve	0.045	0.041	0.043	0.038
Limit of quantification, ng/mL	5	5	5	5

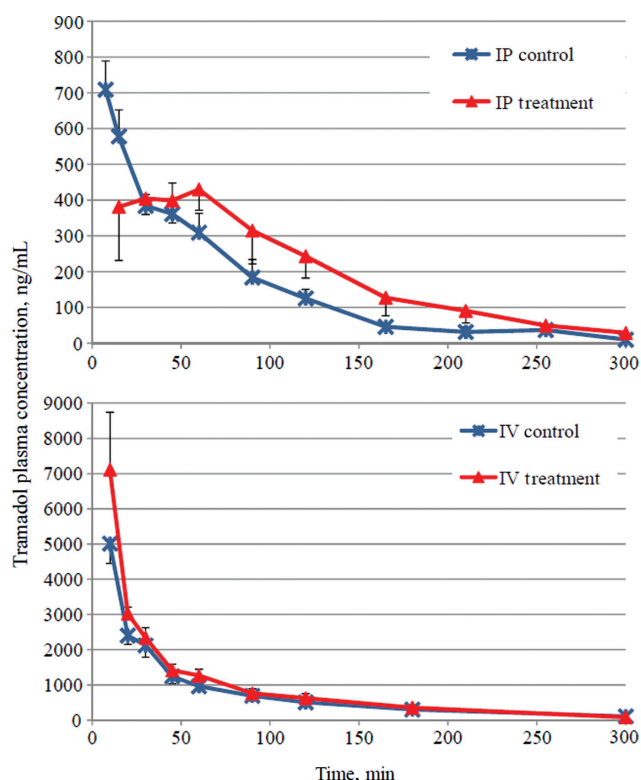


Figure 2: Tramadol (ng/mL) plasma concentration vs. time curves in male Sprague-Dawley rats.

A single dose of 10 mg/kg tramadol was administered via IP (top) or IV (bottom) route. Data are shown as the mean \pm SEM for $n = 6$ rats/group.

to define the tramadol plasma profile in male Sprague-Dawley rats, was a two-compartment, open model with first-order elimination.

The area under the curve (AUC) parameter demonstrates the amount of the compound in the blood. As the obtained data demonstrated in Figure 2 and Table 2, treatment with MDMA produced a 40% and a 120% increase in the $AUC_{(0-20 \text{ min})}$ and $AUC_{(10-300 \text{ min})}$ of the tramadol in the treatment group, respectively. The difference between

Table 2: Pharmacokinetic parameters of tramadol 10 mg/kg in male Sprague-Dawley rats via i.v. or i.p. route in controls (received normal saline) and treatment groups (received MDMA 0.5 mg/kg).

IV	α (min ⁻¹)	β (min ⁻¹)	T _{1/2} α (min)	T _{1/2} β (min)	AUC ₍₁₀₋₃₀₀₎ (min ng/mL)
Control	0.082 ± 0.01	0.01 ± 0.001	9.27 ± 1.19	78.33 ± 13.04	193042.7 ± 28803.0
Treatment	0.144 ± 0.04	0.01 ± 0.001	6.64 ± 1.52	75.56 ± 8.33	231291.7 ± 26961.0
IP	C _{max} (ng/mL)	T _{max} (min)	K _{el} (min ⁻¹)	T _{1/2} (min)	AUC ₍₀₋₃₀₀₎ (min ng/mL)
Control	709.13 ± 80.14	<7.5	0.013 ± 0.003	55.89 ± 10.6	34,934 ± 2618
Treatment	429.83 ± 58.21	60	0.012 ± 0.001	61.92 ± 8.0	55,802 ± 9471

The data are shown as the mean ± SE for n = 6 rats/group.

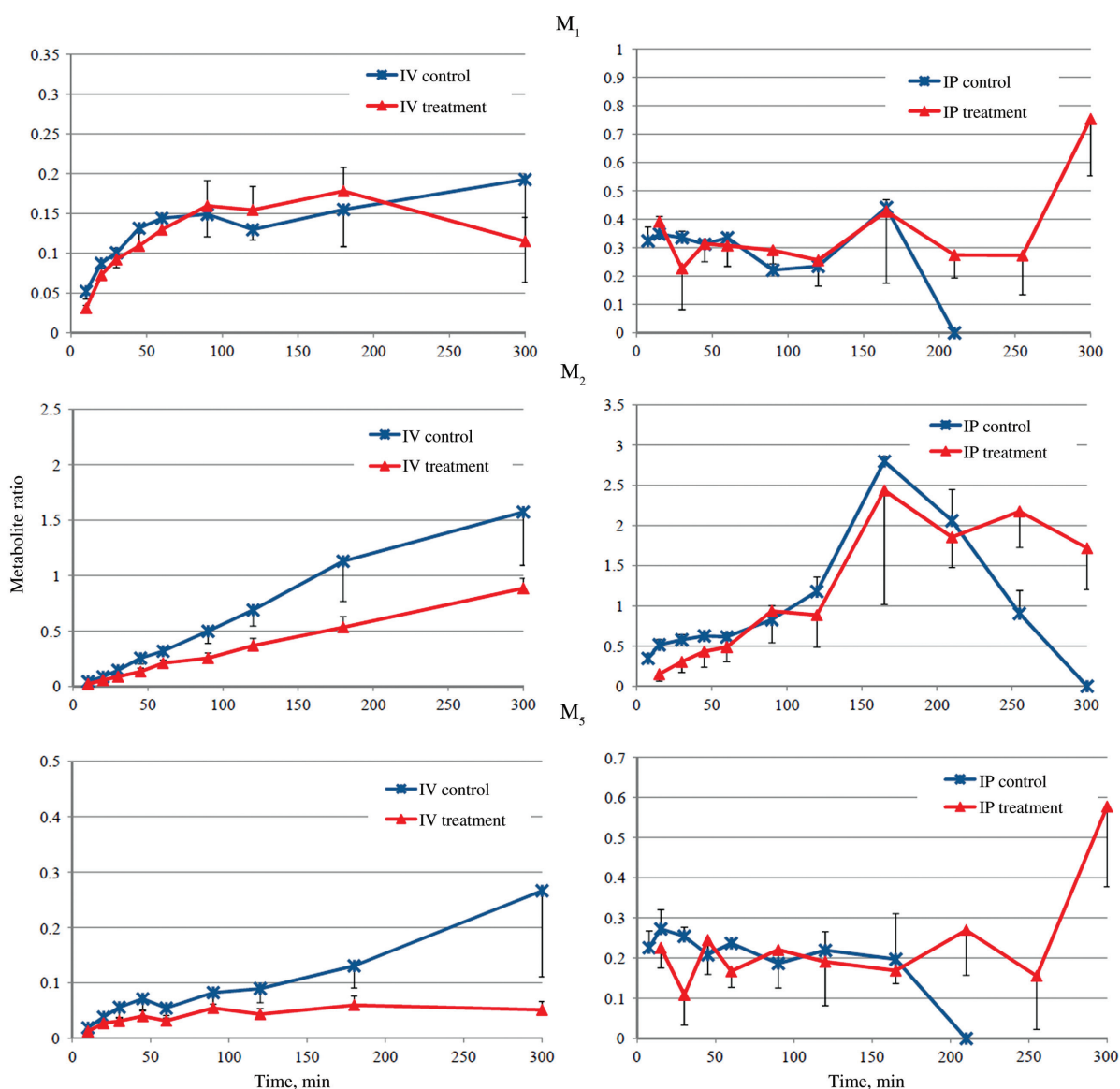


Figure 3: The metabolite ratios of M₁, M₂ and M₅ in the control group (received normal saline) and the treatment group (received MDMA 0.5 mg/kg).

A 10-mg/kg dose of tramadol was administered to male Sprague-Dawley rats via IV (left) or IP (right) route. Data are shown as the mean ± SEM for n = 6 rats/group.

tramadol plasma concentrations in both groups decreased so that at the last points of the plasma profiles, there were no differences between tramadol concentrations in the two groups ($p > 0.05$). The distribution half-life of tramadol was changed from 9.27 ± 1.19 min in the control group to 6.64 ± 1.52 min in the treatment group.

Although MDMA affected the plasma profile of tramadol, and noticeable changes were observed in the distribution phase, it did not produce a significant change in the tramadol half-life (78 ± 13 min in the control group vs. 75 ± 8 min in the treatment group, $p = 0.3$).

The metabolism of tramadol was inhibited by MDMA (Figure 3). Metabolite ratio is used to demonstrate metabolism inhibition. The metabolite ratio of M_1 experienced a 10%–40% reduction in the first 60 min after tramadol administration. This decrease in M_1 production was not statistically significant ($p > 0.9$), whereas a reduction trend in its pattern was seen when compared with the related control group. The production of M_2 , another metabolite of tramadol that is produced mainly via CYP3A4, was affected by MDMA as well. The metabolite ratio of M_2 was reduced by half. The production of M_3 , a metabolite that can be generated from both M_1 and M_2 , was also reduced. The metabolite ratio of M_3 showed about five times reduction at the last point of the plasma profiles.

Tramadol IP administration

In the IP route, the tramadol plasma concentration in control groups was about seven times lower than in the IV route (Figure 2). As data demonstrated in Table 2, the C_{\max} was reduced by 40%, whereas the $AUC_{(0-300 \text{ min})}$ of tramadol showed a 60% increase. These results besides increase in T_{\max} can indicate that the MDMA reduced the rate of absorption of tramadol.

Similar to the IV route, MDMA did not change the tramadol elimination half-life significantly (55.9 ± 10.6 min in the control group vs. 61.9 ± 8.0 min in the treatment group, $p = 0.3$).

The metabolism of tramadol was affected by MDMA. In the control group, the time of last M_1 and M_3 detection was 165 min, whereas in the treatment group, these metabolites were detectable up to 300 min. The rates of tramadol biotransformation to M_1 and M_3 were not changed significantly in the first 165 min (Figure 3). In the case of M_2 , the biotransformation of tramadol to M_2 was decreased in the treatment group. The metabolite ratio reduced to by half in the first 30 min after the IP administration of tramadol, but this difference decreased as time increased.

Discussion

According to our results, MDMA affected the absorption, distribution, and metabolism phases of tramadol but not its elimination rate. In the IP route, MDMA reduced the rate of tramadol absorption. As a result, the T_{\max} was increased, a lower C_{\max} was achieved, and the tramadol metabolites were produced for a longer time. Following IV administration, changes in the distribution phase of the compound were demonstrated. In this phase, passive diffusion, influx, or efflux systems play a major role.

The routine oral dose of tramadol is 50–400 mg/day, which produces a C_{\max} between 70 and 1220 ng/mL among healthy volunteers [3]. The reported plasma therapeutic range for tramadol is about 100–800 ng/mL [27, 28]. If this concentration exceeds 1000 ng/mL, the chance of toxic effects increases [27, 28], and concentrations higher than 2000 ng/mL are considered to be lethal [29]. According to our results, when tramadol was administered via the IV route, the plasma concentration could reach the toxic or lethal range in MDMA abusers. Seizure is the reported adverse effect related to tramadol plasma concentration. A 300-mg IV dose of tramadol has been reported to produce seizures in drug abusers [11]. Serotonin syndrome is another adverse effect that may appear in tramadol users. There is no laboratory test for the diagnosis of this syndrome; the clinical symptoms, such as neuromuscular and autonomic hyperactivity, agitation, excitement, and confusion, can only assist in making the diagnosis [29]. It has been reported that tramadol, along with fluoxetine, paroxetine [7], or venlafaxine [8], which inhibit CYP2D6 and increase serotonin level in the CNS, can cause serotonin syndrome. Because MDMA is reported as a CYP2D6 inhibitor and releaser of serotonin, this adverse drug reaction (ADR) is another probable complication in MDMA abusers.

It was reported by Tzvetkov et al. [30] that tramadol is transported mainly via passive diffusion, whereas this compound and its active metabolite, M_1 , are both substrates for the organic cation transporter 1 (OCT-1). OCTs are influx systems that pump ionic compounds into the cells. Amphoux et al. [31] demonstrated that MDMA is a potent inhibitor of OCT-1 in rats. The reported K_i values (the smaller the K_i , the greater the inhibition) for human OCT-1 and human microsomal CYP2D6 when inhibited by MDMA are 15.0–33.4 and 8.8–46.1 μM [21], respectively. We speculated that the inhibition of OCT-1 or other transporters can increase in T_{\max} and decrease C_{\max} after the IP administration of tramadol in treatment group as well. By decreasing in tramadol absorption and inhibition of metabolism, tramadol metabolites can produce for a longer time.

The metabolism of tramadol is another aspect that is affected by MDMA. Because the $AUC_{(0-300)}$ of tramadol was elevated and the metabolite ratios for two metabolites of tramadol were reduced in both treatment groups, it can be concluded that the metabolism of tramadol was inhibited. Tramadol is mainly converted to its active metabolite, M_1 , by the CYP2D6 isoenzyme; however, M_2 is an inactive metabolite of tramadol that is produced mainly via CYP3A4.

It was confirmed that when MDMA is metabolized via CYP2D6, a main reactive metabolite known as 3,4-dihydroxymethamphetamine is produced, which inhibits its own formation [19, 20]. This inhibition could be either partial or complete based on MDMA concentration, and according to reports, about 250 h is required for 90% recovery of CYP2D6 activity [28, 29]. According to our unpublished and previously reported data [3], the biotransformation of tramadol to M_1 and M_2 is concentration dependent. At high concentrations of tramadol, it seems that M_2 ($K_m = 1021 \mu\text{M}$ [32]) is the major metabolite, and at low concentrations, M_1 ($K_m = 116 \mu\text{M}$ [32]) production is likely greater than M_2 . In this study, M_2 was the major metabolite, and its production decreased in both groups that received MDMA. The reduction in M_1 production was not as much as M_2 . Thus, it can be postulated that MDMA inhibited CYP3A4 more than CYP2D6. This effect is not in accordance with the previously reported study by Yubero-Lahoz et al. [33], which claimed that the inhibitory effect of MDMA was greater on CYP2D6 than CYP3A4 in female subjects. Notably, CYP2D6 activity is higher in female subjects [34], and the reported data in this study were obtained from male rats. It has been reported that methylenedioxy-bearing compounds such as MDMA can inhibit the CYP450 isoenzyme family [35]. Moreover, Heydari et al. [21] proposed that MDMA is likely to inactivate CYP3A4 at high concentrations. To describe the possible cause of the increase in M_1 concentration after the early phase, it can be assumed that the increase may have happened because of the lack of M_1 biotransformation to M_5 .

M_1 exerts its analgesic effects via μ -opioid receptors when the plasma concentration is 20–140 ng/mL [36]. However, this metabolite is responsible for the typical reported opioid-like adverse effects of tramadol, such as nausea, vomiting and respiratory depression [27, 29]. Stamer et al. [37] reported that the response to tramadol analgesia was decreased four-fold in poor metabolizers of CYP2D6, and a deficiency in M_1 production has been postulated as the reason for the poor efficacy of tramadol in this population. Thus, increasing the dose of tramadol is mandatory for better efficacy. By increasing the tramadol dose, the chance of tramadol ADRs is amplified.

M_5 is another metabolite of tramadol that is mainly produced from M_1 and M_2 via CYP3A4 and CYP2D6, respectively. According to our results, the production of M_5 was inhibited in both treatment groups.

In the elimination phase, no statistical differences were observed in the elimination rate constant of tramadol in both treatment and control groups via the IV and IP routes. Because the elimination of tramadol via the kidneys is the prominent elimination pathway (approximately 90%) [3], the inhibition of its metabolism by MDMA does not seem to affect its total elimination rate. However, as a part of the elimination process, the metabolism of tramadol has clearly been affected by MDMA, a change that appeared mainly in the metabolic profile.

Conclusions

Tramadol plasma concentration model in rats was in agreement with the previously reported model for tramadol after IV administration in goats by Cheze et al. [25] and in camels by Ardakani and Rouini [10]. According to our results, MDMA can significantly affect the absorption, distribution, and metabolism of tramadol in an animal model. MDMA can affect the metabolism of tramadol via the inhibition of CYP3A4 and CYP2D6, and it can increase the duration of M_1 production. According to the obtained results, the effect of MDMA on CYP3A4 inhibition may be more significant than that on CYP2D6 inhibition. This finding is important for the compounds that are metabolized through CYP3A4 isoenzymes.

Higher concentrations of tramadol (40%) in plasma during the first 20 min after IV administration of this drug in the MDMA-treated group may lead to ADRs or lethal intoxication. When the route of tramadol administration changes to IP, the absorption rate of this drug is reduced, and a lower C_{max} with longer T_{max} is achieved. Accordingly, to reduce the chance of opiate effects, seizures, and serotonergic syndrome and for better efficacy of tramadol, therapeutic drug monitoring for both tramadol and M_1 is recommended. It should be mentioned that the results of this study need to be clinically supported in human tramadol abusers.

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