Chapter

Revisiting Pharmacokinetics and Pharmacogenetics of Methadone in Healthy Volunteers

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Abstract

Methadone acts as a μ opioid agonist, a serotonin and norepinephrine reuptake inhibitor, and a noncompetitive N-methyl-D-aspartate receptor antagonist. These actions altogether are responsible for its efficacy in the management of chronic pain. It is available as a racemic mixture of (R)- and (S)-methadone, both being stereoisomers responsible for its analgesic effect. Methadone elimination occurs mainly through metabolism in the liver by CYP3A4, CYP2B6, and CY2C19 and to a lesser extent by CYP2D6 and in the intestine by CYP3A4. The relative intestinal content of CYP2B6 and CY2C19 is unknown but it seems that CYP2B6 is not present at the intestine. CYP3A4, CYP2B6, and CYP2C19 convert methadone mainly into 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine(EDDP). CYP2B6 and CYP2C19 are stereoselective to S- and R-enantiomer, respectively. The pharmacokinetic study carried out in healthy volunteers by our research group confirmed that MTD undergoes recirculation via gastric secretion and intestinal reabsorption and revealed that the drug is extensively metabolized in the liver but intestinal metabolism is not only relevant but also stereoselective. Polymorphisms of the CYP2B6 and CYP2C19 isoenzymes and their relationship with the pharmacokinetics of MTD were also assessed.

Keywords: methadone stereoisomers, EDDP stereoisomers, pharmacokinetics, pharmacogenetics, stereoselectivity

1. Introduction

Methadone (MTD) is a synthetic opioid with primarily a μ and δ opioid agonist action, but some other novel mechanisms implied in pain relief such as antagonism of the N-methyl-D-aspartate (NMDA) receptor, and inhibition of serotonin and norepinephrine reuptake are also reported in the literature [1–5]. These multiple receptor activities make it an attractive choice for analgesia. It is increasingly used to manage cancer and chronic nonmalignant pain [6, 7] and although some authors stated its use in neuropathic pain as well, [3, 8, 9] good evidence for this use is still lacking [10]. NMDA antagonism has an important role in attenuating tolerance [11].

In comparison to oral morphine and other opioids, MTD has a higher bioavailability and initial rapid and extensive distribution and a slower elimination rate.

Unfortunately, it is the unique pharmacokinetics and pharmacodynamics of MTD that render its somewhat unpredictable effects.

This chapter focuses on revising plasma-gastrointestinal-plasma recirculation of MTD, evaluating the relative importance of CYP3A4, CYP2B6, and CYP2C19 isozymes in the metabolism of the drug, and assessing the possibility of attenuating the metabolism mediated by localized isoenzymes, mainly in the liver to favor the recirculation of the drug. Polymorphisms of the CYP2B6 and CYP2C19 isoenzymes and their relationship with the pharmacokinetics of MTD in healthy volunteers are also dealt with.

2. Pharmacokinetic study

MTD is a racemic mixture of two enantiomers: (S)-methadone and (R)-methadone. (R)-methadone accounts for its opioid effect with a minor antagonism on NMDA-receptors, whereas (S)-methadone is responsible for serotonin and norepinephrine reuptake inhibition and NMDA-receptor antagonism [4, 5, 12–14].

The mean bioavailability of MTD is around 75% (range 36–100%). MTD undergoes first-pass metabolism and is detected in plasma 30 minutes after intake. The time needed to reach peak concentration in plasma (Tmax) in patients is 4.4–6 hours and 2.8 hours in healthy volunteers [15, 16]. It is also an efflux transporter (P-glycoprotein) substrate [17]. MTD is a highly lipophilic drug with basic properties (pKa = 8.3) [18]. Following absorption, it is distributed to the brain, liver, kidneys, lungs, and muscles. It binds to alpha-1-acid glycoprotein (60–90%) [19]. The fluctuation in the levels of this protein with physiological and pathological changes and with age and sex explains the variability in plasma protein binding of basic drugs both between individuals and within individuals [20]. (R)-MTD has lower plasma protein binding in comparison with the (S)-enantiomer [21].

The metabolism of MTD is thought to occur mainly in the liver by the cytochrome P450 (CYP450) enzyme system, primarily by CYP3A4 also located in the intestine, but human drug-drug interaction studies are not consistent with this and other enzymes are thought to be more involved in its hepatic metabolism such as CYP2B6 and CYP2C19. Excretion through the kidneys and feces is not negligible and since MTD is a basic drug, if urinary pH increases, MTD clearance in urine decreases [22]. Its principal metabolite is N-demethyl MTD which rapidly converts into 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP). The hierarchy of transforming MTD into EDDP is CYP2B6 > CYP2C19 ≥ CYP3A4. CYP2B6 is responsible mainly for metabolizing the (S)-enantiomer, while CYP2C19 shows preference for the (R)- enantiomer. CYP3A4 shows no enantioselectivity [23–25]. The isoenzyme CYP2D6 is also implicated in metabolizing MTD but through a different pathway and to a lesser extent [26]. The unbound MTD clearance is stereoselective, being the S-enantiomer cleared faster [21].

The elimination half-life after the first dose is longer than at steady state due to induction of CYP3A4 and P-glycoprotein by MTD [15, 16, 27]. Our research group found a nonlinear relationship between steady-state MTD plasma concentrations and daily dose [28].

Due to its basic properties, MTD can be recovered in gastric juice [29] and subsequently reabsorbed after the gastric content is emptied into the duodenum completing a blood-gastrointestinal-blood recycling.

Although venous plasma drug concentrations are the ones used in pharmacokinetics studies, vein and artery drug concentrations are not the same throughout time. Arterial drug concentrations are higher than the respective venous concentrations during drug input. For highly lipophilic drugs rapidly distributed from

arterial blood to tissues such as MTD, such increased tissue/venous plasma ratio would explain the toxicity of MTD in certain tissues and the lack of correlation between venous MTD concentrations and adverse effects [15]. When elimination predominates, the opposite is observed [30]. However, if MTD recycling is operating at the monoexponential decay of levels, increased arterial/venous plasma drug concentration ratios will also be observed due to drug reabsorptions.

So its storage in body tissues and the slow release to plasma as well as its recycling process could be responsible for its prolonged elimination half-life. This last fact is exploited in preventing withdrawal symptoms. However, the long half-life does not seem to correlate with the observed shorter duration of analgesia (6–12 hours) after steady state is reached [31].

As measuring drug levels in arteries is an uncommon practice, our group has been working for a long time [32–35] using saliva in order to surrogate arterial free plasma drug concentrations as this biological fluid highly correlates with arterial plasma due to the fact that it is produced by ultrafiltration of the latter [36]. Salivary peaks during the elimination phase would be indicating reentry processes as it was observed in a study carried out with patients [35].

It is important to study the stereoselectivity of MTD metabolism once the blood-gastrointestinal tract cycling is operating, and to investigate whether the intestinal metabolism of MTD could be assessed as relevant in relation with the hepatic one. For this purpose, our research group has carried out an *in vivo* study.

2.1 Subjects and study design

An *in vivo* randomized, single-dose, crossover, and compensated study with two periods and two treatments (A and B) was carried out. A single dose (10 mg) of MTD was administered to 12 healthy volunteers (six women and six men between 18 and 42 years old) under fasting conditions. Blood, saliva, and urine samples were taken to determine pharmacokinetic and exposure parameters for both enantiomers of MTD and of its main metabolite (EDDP), as well as for the genotyping studies. The previous night and 30 minutes before the administration of MTD, the subjects received a dose of 10 mg of metoclopramide in order to avoid nauseas and vomits. Part of these results has already been published [37].

Food intake was standardized in the study protocol and was different for treatments A and B. There was a higher frequency of food intake in the latter in order to investigate the impact of blood-gastrointestinal tract-blood recirculation processes on MTD metabolism. In treatment A, volunteers received lunch, dinner, and breakfast at 4, 13, and 24 hours post dose, while during treatment B, the volunteers received lunch, a light meal, a snack, dinner, and breakfast at 4, 7, 10, 13, and 24 hours post dose. Only frequency of food intake differs between treatments A and B.

The study conformed to standards indicated by the Declaration of Helsinki and its later amendments, approval was provided by the Ethics Committee of the Faculty of Chemistry (Uruguay), and all healthy volunteers in the study gave written informed consent prior to participation.

2.2 Sampling and MTD and EDDP determination

Blood samples were withdrawn from the antecubital vein through cannulation and saliva samples were collected in Salivette® tubes at the following times: 0–0.5–1–2–3–4–6–8–10–12–16–24–36–48–72 and 96 hours post dose. Urine was collected at 0 (before dose intake) and at the end of the following intervals: 0–2, 2–4, 4–7, 7–8.5, 8.5–10, 10–11.5, 11.5–13, 13–14.5, 14.5–16, and 16–24 hours after dosing and sample volumes were recorded. Aliquots of urine samples were kept in order to

measure the analyte content. Immediately after sampling, pH was measured using a portable pH meter for urine samples. All samples were kept in a freezer at −25°C until the time of analysis.

When the pre-dose blood sample was taken, another blood sample was taken to obtain genomic DNA in order to determine the genotype of the CYP2B6 and CY2C19 isoenzymes of the subjects.

MTD enantiomers in plasma, saliva, and urine were quantified. EDDP enantiomer quantification was performed in urine. MTD and EDDP were extracted with a mixture of hexane and isoamyl alcohol from 2.0 mL of plasma or 1.0 mL of urine or saliva samples that were previously alkalinized. Then, the organic phase was evaporated under a stream of nitrogen, and the residue was reconstituted with the mobile phase. Imipramine (10.00 μg/mL) was used as the internal standard and 50 mL was added to plasma or urine or saliva. MTD (in all the three fluids) and EDDP (only in urine) quantification was performed using a validated HPLC-UV chiral method, which was an adaptation of a previously published methodology [38]. The mobile phases consisted of phosphate buffer 20 mM pH 6.0 + 2 mM diisopropylamine: acetonitrile (92:8) for urine analysis and phosphate buffer 20 mM pH 7.0 + 2 mM diisopropylamine: acetonitrile (82:18) for plasma and saliva analysis. The flow rate was 0.7 mL/min. The separation of the compounds was performed on a CHIRALPACK AGP™ (100 × 4 mm; 5 μm) column with a silica guard column. Detection was performed at a wavelength of 215 nm. The analysis was carried out at 25°C and the injection volume was 80 μL.

The HPLC method was linear for MTD between 4.0 and 160 ng/mL and between 19.0 and 3280 ng/mL for plasma or saliva and urine samples, respectively. The linearity for EDDP in urine was proven from 52.0 to 4200 ng/mL. Inter- and intra-day precision and accuracy were below 14% for both compounds.

2.3 Pharmacokinetic and statistical analysis

The following pharmacokinetic parameters were obtained from the MTD plasma and saliva concentration versus time curves for both enantiomers of MTD:

- Cmax: Maximum concentration.
- Tmax: Time to maximum concentration.
- AUC [0–96]: Area under the concentration-time curve from 0 to 96 hours.
- AUC [0–24]: Area under the concentration-time curve from 0 to 24 hours.
- R/S: Concentration ratio of the enantiomers.

Experimental Cmax and Tmax were computed and the AUC was estimated by the trapezoid method up to 96 hours, or until the last quantifiable concentration time. As for most of the subjects, the concentrations were not quantifiable for times longer than 24 h and AUC was determined up to 24 h. The R/S concentration ratio was computed as an indicator of possible stereoselective metabolic changes because of drug recycling.

From the urinary concentrations of MTD and EDDP and the volumes of urine recorded, the amounts excreted in the time interval between two consecutive micturitions were calculated. Excretion rates versus time were plotted and the R/S ratios of MTD and EDDP were calculated for this parameter.

Statistical significances between means were assessed by a nonpaired (between sexes) and a paired (between enantiomers) t-student test.

2.4 Results and discussion

Mean R- and S-MTD plasma concentration-time profiles for treatments A and B in women and men are shown in **Figure 1**. As it is shown in this figure and in **Table 1**, a higher exposure of S-MTD for both treatments can be observed due to its higher plasma protein binding.

Figure 1 also shows a secondary peak 8 hours post dose (4 hours post lunch). This means a re-entry of the drug into the bloodstream, as a consequence of a plasma-gastrointestinal-plasma recirculation process of MTD.

The feasibility of MTD to follow this recirculation process is due to its basic nature previously mentioned. MTD can be secreted into the gastric juice as a consequence of the pH gradient between plasma (pH = 7) and gastric juice (pH = 1.2). In addition, after food intake there is an increase in blood flow and in the fraction of cardiac output destined to the gastric area, which would favor the secretion of MTD to the gastric juice. When food reaches the stomach, several milliliters of gastric juice are poured into the gastrointestinal tract, so molecules of MTD that could have been secreted into the gastric juice from the blood would pass into the intestinal lumen and could be re-absorbed from there again, re-entering the bloodstream. This secondary peak was evidenced in the sample obtained 8 hours post dose for both treatments, but the process could have begun sometime before as a result of food intake and depending on the gastric emptying of each volunteer. No differences were observed in the appearance of secondary peaks between treatments A and B, so a higher frequency of food intake does not add more mass of recirculating molecules, but perhaps a prolongation of the recirculation process.

Table 1 summarizes the results obtained from the plasma samples.

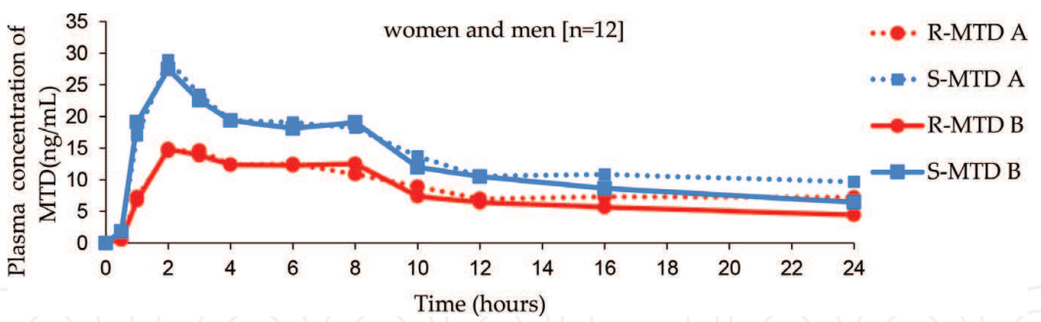

Figure 1.
Mean R- and S-MTD plasma concentration-time profiles for treatments A and B in women and men.

		$t_{1/2}$ ± SD (h)		AUC (0–24) ± SD (ng.h/mL)		C_{MAX} ± SD (ng/mL)		T_{MAX} (range)[a] (h)	
		R	S	R	S	R	S	R	S
Plasma	Women	27.3 ± 12.6	22.1 ± 7.8	190[b] ± 61	302[b] ± 89	18.6[b] ± 10.3	32.2[b] ± 8.4	3.5 (2.0–8.0)	2.0 (1.0–8.0)
	Men	25.0 ± 4.7	24.6 ± 6.8	192[b] ± 101	304[b] ± 118	17.5[b] ± 7.9	31.0[b] ± 6.3	3.0 (1.0–10)	2.0 (1.0–8.0)

[a]*median (range).*
[b]*$p < 0.01$, paired t-student test between R and S.*

Table 1.
Mean (± standard deviation) pharmacokinetic parameters of MTD obtained in women and in men.

The value of Tmax obtained in healthy volunteers is in agreement with the literature [16, 27]. In patients, the value of Tmax is higher in comparison to healthy volunteers as chronic use of MTD delays gastric emptying and gastric motility and hence absorption.

Urinary exposure, as can be seen in **Figure 2**, showed an inverse relationship between isomers. Bearing in mind that the rate of urinary excretion of MTD could subrogate its free plasma concentration, a lower intrinsic clearance of the R-isomer could be evidenced and therefore a stereoselective biotransformation in favor of the S-MTD.

Volunteers excreted significantly ($p < 0.01$) more (R)-methadone and (S)-EDDP ($p < 0.001$) than the corresponding enantiomers as is shown in **Figures 2** and **3**, respectively. However, as information about the stereoselectivity of the metabolite clearance is lacking, no conclusion can be drawn about its bioavailability.

The profile of the urinary excretion rate of MTD did not show the same pattern of secondary peaks as the profile of MTD plasma concentrations did. This could be explained by a significant drop in the rate of excretion after lunch, which can be attributed to the well-known increase in urinary pH after food intake (postprandial alkaline tide), causing a decrease in urinary MTD excretion.

A higher incidence of nausea was detected in women than in men during the experimental phase of the study; in fact, this adverse effect was not observed in men. This motivated a differentiated analysis of the results according to the sex of the subjects, as differences in the pharmacokinetics of opioids between the sexes can affect the safety and efficacy of the treatments. The pharmacological activity can be better predicted from free plasma concentrations than from the total ones, and as mentioned above, the rate of urinary excretion of MTD could subrogate the free plasma concentration. Women presented a higher urinary exposure of R-MTD (mainly responsible for the μ effect), which correlates with the greater intensity of adverse effects that they presented around Tmax in comparison to men. This is also shown in the profiles of saliva concentrations of MTD (**Figure 4**), which are also related to free plasma concentrations.

To assess stereoselectivity in MTD metabolism, R/S ratios were studied throughout time as is shown in **Figure 5**. R/S ratios of MTD were constant once absorption

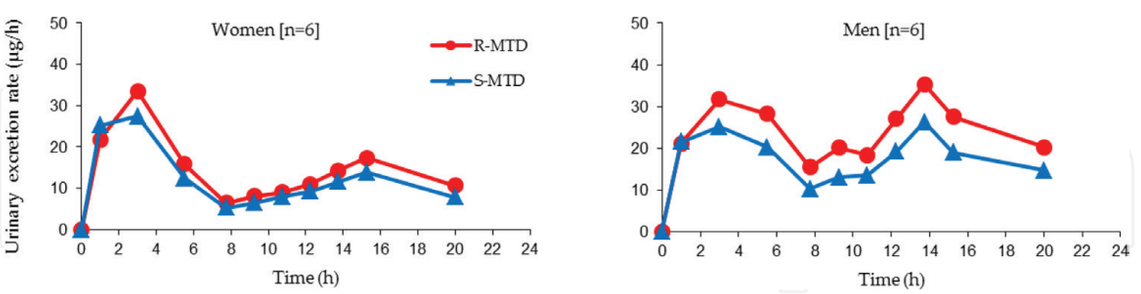

Figure 2.
Mean urinary excretion rates of R- and S-MTD versus time in men and women.

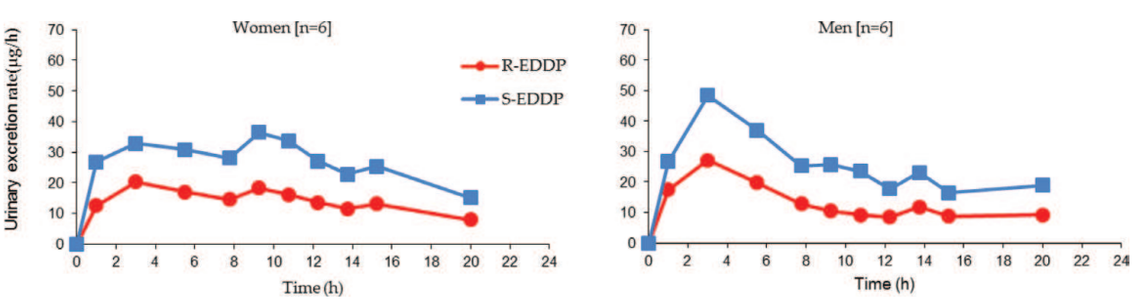

Figure 3.
Mean urinary excretion rates of R- and S-EDDP versus time in men and women.

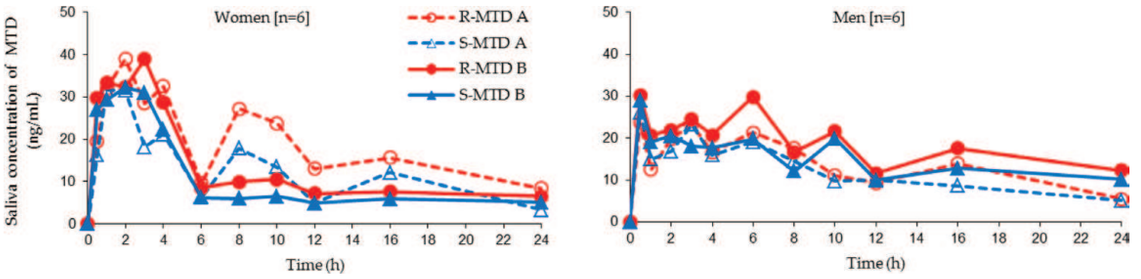

Figure 4.
Mean R- and S-MTD saliva concentration-time profiles for treatments A and B in women and men.

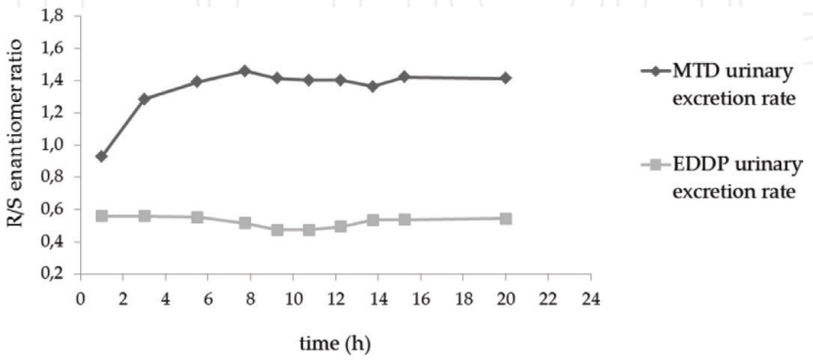

Figure 5.
Mean (± 95%CI) R-to-S MTD and EDDP urinary excretion rate ratios after oral administration of MTD.

had finished. During the absorption and rapid disposition phase, this ratio is increasing. However, R/S ratios of EDDP were constant from the beginning, except after food intake (mainly between 3 and 7 hours post lunch intake when MTD recirculation is taking place) when the ratio decreased and this might explain differences in EDDP systemic formation.

The molecules of MTD present in the systemic circulation undergo both intestinal and hepatic stereoselective metabolism by CYP2C19 and CYP2B6 enzymes. CYP2C19 is stereoselective towards the R-isomer while CYP2B6 towards the S-isomer. After food intake, when a process of drug reentry is operating, the molecules of MTD that had been secreted into the gastric juice can be reabsorbed in the intestine. Consequently, a greater number of molecules enter the enterocyte. The change observed in the R/S ratio of EDDP after the ingestion of meals evidences a different stereoselectivity between intestinal and hepatic metabolism, possibly due to a relative differential content of CYP3A4 and CYP2C19 in enterocytes and hepatocytes, being the relative presence of CYP3A4 greater at the intestine. In the case of MTD, the metabolism of the S-enantiomer is favored after the passage of MTD through the intestine compared to its passage through the liver. Although during food intake there is an increased blood flow to the splanchnic area, and the liver and the other organs in this region receive a greater number of molecules from the blood coming from areas that do not belong to the splanchnic region, for drugs secreted in the gastric juice, the fraction of molecules that the intestine receives is even greater because there is a supplementary quantity of molecules that enter the intestine coming from the gastric juice. If no secretion was taking place, the molecules would be transferred from the stomach directly to the liver through the portal bloodstream without passing through the enterocytes.

Therefore, by favoring recirculation rather than bypassing hepatic metabolism, the intestinal metabolism would be increasing. Our research reveals an important role of the intestine in the systemic (and pre-systemic) metabolism of MTD, presenting a greater stereoselectivity towards the S-isomer. Although this isomer has little or no activity as an opioid agonist, it is able to inhibit the reuptake of

serotonin and noradrenaline, in addition to acting as a noncompetitive antagonist of NMDA receptors, actions that enhance the opioid analgesic effect of the R- isomer. As a result, by favoring recirculation, the analgesic potency of MTD would not be increasing but decreasing instead. This could explain the shorter duration of the analgesic effect of MTD in view of the reported long elimination half-life.

3. Pharmacogenetic study

CYP2B6 and not CYP3A4 is the principle determinant of clinical MTD elimination and is one of the most polymorphic cytochrome P450 (P450) genes in humans and, currently, it has 30 defined alleles with over 100 described polymorphisms [39]. According to Kharasch et al. [40], CYP2B6*6 allele carriers showed higher MTD concentrations and slower elimination, whereas CYP2B6*4 carriers had lower concentrations and faster elimination.

CYP2C19 plays an important role in MTD metabolism and CYP2C19 gene is highly polymorphic as well. Loss of enzyme activity results from the CYP2C19*2 allele and the CYP2C19*17 allele is associated with increased enzymatic activity [41, 42].

3.1 Methodology

Once the genomic DNA was obtained from the leukocyte fraction, the individuals were genotyped for the CYP2B6 and CYP2C19 genes by massive sequencing, which was carried out at the Institute of Genomic Medicine (INMEGEN) in Mexico.

In order to be processed by massive sequencing, genomic DNA samples should have a concentration higher than 10 ng/μL, and the ratio of absorbances 260/280 and 260/230 should be approximately 2 to be able to consider that the DNA obtained was of good quality. In cases in which the sample did not meet these requirements, purification was performed using the Mag Jet Genomic DNA Kit (Thermo Scientific) which includes incubation with proteinase and RNAse and purification with magnetic beads.

As a result of this processing, the genotype of the 12 volunteers was obtained for CYP2B6 and CYP2C19 enzymes. Considering the polymorphisms found and based on the literature, we determined the phenotype that would be expected, that is, increased, normal, or decreased enzyme activity.

3.2 Results and discussion

Regarding the polymorphisms in the gene that encodes CYP2C19, 5 of the volunteers in our study presented the allelic variant * 2 (rs4244285), which is associated with a decrease in the activity of the enzyme, whereas 2 volunteers presented the allelic variant * 17 (rs3758581), which is associated with an increase in the activity. Regarding the polymorphisms in the gene that encodes CYP2B6, 6 volunteers presented the allelic variant * 4 (rs2279343), which determines an increased enzymatic activity.

S/R ratios for MTD in plasma and urine and the S/R ratios for EDDP in urine were calculated. The individuals were grouped into two. Group 1 included those volunteers in whom the activity of CYP2B6 was increased and CYP2C19 activity was normal or decreased as well as those volunteers in whom CYP2B6 activity was normal but CYP2C19 activity was diminished. Group 2 included those individuals with normal activity of both enzymes as well as those in which the activity of CYP2B6 was normal but that of CYP2C19 was increased and a case

Group	Volunteer	CYP2B6 activity	CYP2C19 activity	S/R MTD in plasma	S/R MTD in urine	S/R EDDP in urine
Group 1	Vol. 1	Increased	Decreased	1.63	0.840	1.757
	Vol. 2	Increased	Decreased	1.09	0.684	1.992
	Vol. 4	Increased	Normal	1.80	0.949	1.705
	Vol. 5	Increased	Decreased	1.65	0.818	2.019
	Vol. 8	Normal	Decreased	1.84	0.732	1.751
	Vol. 9	Normal	Decreased	1.61	0.782	2.050
	Vol. 11	Increased	Normal	1.55	0.567	1.707
Group 2	Vol. 3	Increased	Increased	1.55	0.703	2.071
	Vol. 6	Normal	Normal	1.43	0.869	1.729
	Vol. 7	Normal	Increased	1.89	0.846	2.011
	Vol. 10	Normal	Normal	1.86	0.743	2.277
	Vol. 12	Normal	Normal	1.51	0.528	
Average of the total number of volunteers				1.62	0.76	1.92
Standard error				0.065	0.032	0.058
Average Group 1				1.60	0.77	1.85
Standard error Group 1				0.093	0.046	0.060
Average Group 2				1.65	0.79	2.02
Standard error Group 2				0.094	0.036	0.113

Table 2.
S/R ratios for MTD in plasma and urine and for EDDP in urine obtained in treatment A and the activity of CYP2B6 and CYP2C19.

in which the activity of both enzymes was increased. This classification allowed grouping those individuals, in whom a preferential biotransformation was expected on the S isomer, considering the activity of the enzyme together with its stereoselectivity. The averages of the S/R ratios for each group were calculated, both for treatment A and for treatment B, and the results are shown in **Tables 2** and **3**, respectively.

The three average S/R ratios were compared by a t-student test, and no significant differences were obtained in any of the cases. However, the S/R ratios of MTD either in plasma or in urine are lower in Group 1 compared to Group 2, which is in agreement with the stereoselectivity of CYP2B6 towards the S-MTD since the metabolism of the S-isomer is greater compared to the R-isomer when the activity of CYP2B6 is increased and the activity of CYP2C19 decreased. The results obtained for the S/R ratios of EDDP are different, probably because the biotransformation of MTD mediated by these enzymes also leads to the formation of other metabolites. Moreover, there is a lack of information in the literature about the stereoselectivity of EDDP clearance.

Genetic variation of CYP2C19 mainly affects MTD metabolism, and it has a minor effect on the metabolite, maybe because it contributes very little to EDDP formation (1/10 compared to CYP2B6 contribution).

Group	Volunteer	CYP2B6 activity	CYP2C19 activity	S/R MTD in plasma	S/R MTD in urine	S/R EDDP in urine
Group 1	Vol. 1	Increased	Decreased	1.74	0.874	1.534
	Vol. 2	Increased	Decreased	1.26	0.716	1.848
	Vol. 4	Increased	Normal	1.41	0.958	1.565
	Vol. 5	Increased	Decreased	1.83	0.776	1.993
	Vol. 8	Normal	Decreased	1.63	0.717	1.657
	Vol. 9	Normal	Decreased	1.53	0.749	2.397
	Vol. 11	Increased	Normal	1.77	0.638	1.923
Group 2	Vol. 3	Increased	Increased	1.37	0.820	2.126
	Vol. 6	Normal	Normal	1.86	0.866	1.871
	Vol. 7	Normal	Increased	1.71	0.781	1.635
	Vol. 10	Normal	Normal	1.65	0.766	1.961
	Vol. 12	Normal	Normal	1.95	0.580	
Average of the total number of volunteers				1.64	0.77	1.86
Standard error				0.061	0.027	0.078
Average Group 1				1.60	0.78	1.85
Standard error Group 1				0.078	0.041	0.114
Average Group 2				1.71	0.81	1.90
Standard error Group 2				0.100	0.020	0.102

Table 3.
S/R ratios for MTD in plasma and urine and for EDDP in urine obtained in treatment B and activity of CYP2B6 and CYP2C19.

4. Conclusions

Our results confirm MTD recirculation via gastric secretion and subsequent intestinal reabsorption. MTD is extensively metabolized in the liver but intestinal metabolism is not only relevant but also stereoselective.

Although the opioid effect of MTD is mainly due to the R-isomer, the S-isomer also has an analgesic action by inhibiting the reuptake of serotonin and noradrenaline and by exhibiting a noncompetitive antagonism of the NMDA receptor. The latter action is also responsible for preventing or attenuating tolerance and withdrawal syndrome. Therefore, in those patients who have an increased activity of the CYP2B6 enzyme or a normal activity of this enzyme in combination with a decreased activity of CYP2C19, (situations that favor the S-isomer metabolism), the analgesic effect could be diminished and the development of tolerance as well as the withdrawal symptoms could be exacerbated.

Despite the fact that blood-gastrointestinal-blood recycling extends the residence of a drug in the body, in this case, the elimination of the S-isomer is increased with each passage through the enterocyte. Consequently, the recycling process of MTD would not be favoring an increased analgesic effect as it would be expected. This is in agreement with the shorter duration of analgesia observed in the clinical setting after steady state is reached.

The occurrence of frequent adverse effects such as nausea was observed only in women, even after receiving two doses of metoclopramide prior to the dose of MTD. Although tolerance to nausea and vomits develop with chronic use, the physician should consider a lower starting dose of 5 mg/day for women. Apparently, an initial dose of 10 mg/day for men could be appropriate.

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Conflict of interest

The authors declare no conflict of interest.

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