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Original Communication

Stereoselective metabolic change of methadone caused by its blood-gastrointestinal cycling

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ABSTRACT

We focused our investigation on methadone (MTD) because its uses are now gaining importance in the treatment of chronic pain. Six women and six men were given a single oral dose of 10 mg of MTD under fasting conditions. Following the administration of MTD, two meals were given at 5 and 13 hours post-dose. Plasma and urine samples were collected, and MTD and its main metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP, only in urine) were stereoselectively monitored by means of high-performance liquid chromatography (HPLC) method. Both in men and in women, R-MTD showed a lower plasma exposure than S-MTD because of its lower plasma protein binding. Urinary exposure showed an inverse relationship. The higher R-MTD urinary concentrations found in women could explain the more intense side effects they experienced in relation with men. Secondary peaks were observed in both sexes after meal intake, revealing that MTD follows blood-gastrointestinal tract recirculation. Its basic properties enable its secretion to the gastric juice, and thereafter its reabsorption from the intestinal lumen. Interestingly, a significant decrease in the R-to-S EDDP excretion rate ratio after food intake was observed. This finding could be due to a difference in the stereoselective enantiomer metabolism between the enterocytes and the hepatocytes, in favour of S-MTD, due to a plausible difference in enzyme ratio between intestine and liver. Men showed a non-significant higher decrease in the R-to-S EDDP ratio. These findings evidence not only that the intestinal metabolism of MTD is relevant, but also that it is stereoselectively different from the hepatic one. MTD would not be favoured from gastrointestinal tract recycling, especially the S-enantiomer, because every time it passes through the enterocytes its elimination increases.

KEYWORDS: methadone stereoisomers, EDDP stereoisomers, blood-gastrointestinal tract recirculation, intestinal metabolism.

ABBREVIATIONS

MTD, Methadone; NMDA, N-methyl-D-aspartate; S/P, saliva/plasma; EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; GIT, gastrointestinal tract; P-gp, P-glycoprotein.

1. INTRODUCTION

Methadone (MTD) is a synthetic opioid used for oncologic and non-oncologic pain treatment and as a substitution treatment agent for patients in addiction to heroin recovery [1]. MTD is commonly administered as a racemic mixture. The R-enantiomer is responsible for the effect over μ and δ receptors and also antagonizes the N-methyl-D-aspartate (NMDA) receptor. The S-enantiomer is not only an NMDA receptor antagonist but also inhibits the norepinephrine and serotonin reuptake [2, 3].

MTD is a highly lipophilic compound with basic properties (pKa = 8.3) [4]. Because of this, it can be recovered in gastric juice [5]. The accumulated

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drug is available for reabsorption after gastric content is emptied into the duodenum. Re-entry processes can be detected during the elimination phase through changes in the saliva/plasma (S/P) concentration ratio, which increases after food intake [6-9].

Its elimination occurs mainly through metabolism (76%). Various enzymes from the cytochrome P450 system are involved. Its principal metabolite is N-demethyl MTD which rapidly converts into 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP). Only isoenzymes CYP2B6, 2C19 (both located mainly in the liver) and 3A4 (located in both the liver and the intestine) biotransform MTD into EDDP [1, 10-16]. CYP2B6 metabolizes preferably the S-enantiomer. CYP2C19 shows preference for R-enantiomer, while CYP3A4 shows no enantioselectivity [1, 17, 18]. The isoenzyme CYP2D6 (located mainly in the liver) also metabolizes MTD but through a different route. The amount of MTD excreted in urine (24%) is pH dependent [1, 10].

MTD is mostly bound to alpha-1 acid glycoprotein. Compared with the S-enantiomer, R-MTD has lower plasma protein binding. The unbound MTD clearance is stereoselective, being the S-enantiomer more cleared [19]. Kristensen *et al*. [20] reported a mean plasma clearance of R-MTD (158 mL/min) significantly greater than that for S-MTD (129 mL/min), while the elimination half-life of S-enantiomer was lower (28.6 versus 37.5 h). Elimination half-life is longer after the first dose than during maintenance treatment [17, 21]. MTD is capable of inducing its own metabolism through the induction of the isoenzyme CYP3A4 [21] and/or efflux transporter, P-glycoprotein (Pgp). The higher S/P concentration ratio and gastric concentration of MTD observed in chronic patients evidence the auto-induction of Pgp at salivary and gastric levels [5].

The main objective of the study was to detect if any changes in the stereoselective pattern of MTD metabolism might happen once the blood-gastrointestinal tract (GIT) cycling was activated by food intake, and to investigate whether the intestinal metabolism of MTD could be assessed as relevant in relation with the hepatic one.

To accomplish these goals, MTD isomers were monitored in plasma and urine. Stereoselective changes in MTD metabolism were followed through the determination of the parent drug and R- and S-EDDP in urine.

2. MATERIALS AND METHODS

2.1. Subjects and study design

Six women and six men between 18 and 42 years old with mean body weight (± SD) of 52.8 (± 6.6) and 68.8 (± 9.6) kg, respectively were enrolled in a two-way crossover study, where single oral doses of 10 mg of MTD (Lazar Laboratories, Montevideo, Uruguay) were given under fasting conditions (last meal ingested at least 8 h before dosing). The dose was chosen from a previously reported study where a single dose of 0.2 mg/kg was administered [22]. Also, our group has experience working jointly with the Pain Unit Service, and a 10 mg daily dose of MTD is the lowest dose used by the physicians [8].

Subsequent to the administration of MTD, two meals were given at 5 and 13 hours post-dose. Lunch (5 h post-dose) consisted of a steak sandwich, a dairy dessert and a glass of water. Dinner (8 h after lunch) consisted of pasta, a fruit and a glass of water. Thirty minutes before dosing, 10 mg of metoclopramide were administered in order to prevent nausea and vomiting, a very frequent side effect observed after the administration of MTD [1].

The study was conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki), and the Institutional Ethics Review Committee of the Faculty of Chemistry (Uruguay) approved the experimental protocol. Written informed consent was obtained from all subjects before their entry into the study. The study was performed in the Bioavailability and Bioequivalence Centre for Medicine Evaluation (*CEBIOBE* as abbreviation of its Spanish spelling), situated in “Dr. Juan J. Crottogini” Hospital (Montevideo, Uruguay).

2.2. Sampling and chemical analysis

Blood samples were withdrawn from the antecubital vein through cannulation, at 0 (before dose intake) and 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36, 48 hours after dosing, and immediately transferred into heparinized tubes. Plasma was then separated by centrifugation. 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, 16-24 hours after dosing. Aliquots of urine samples were kept in order to measure their analyte content. Immediately after sampling, pH was measured using a portable pH meter for urine sample. All specimens (plasma and urine) were stored at -25 ºC until the quantification of analytes.

Sample preparation involved extraction of MTD and EDDP with a mixture of hexane and isoamyl alcohol from 2.0 mL of plasma or 1.0 mL of urine samples previously alkalinized. The organic phase was then evaporated under a stream of nitrogen and the residue was reconstituted with mobile phase. Fifty microliters of imipramine solution (10.00 μg/mL) was used as internal standard. MTD (in both 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 [23]. Mobile phases consisted of phosphate buffer 20 mM pH 6.0 + 2 mM diisopropyl amine:acetonitrile (92:8) for urine analysis and phosphate buffer 20 mM pH 7.0 + 2 mM diisopropyl amine:acetonitrile (82:18) for plasma analysis. Flow rate was 0.7 mL/min. A CHIRALPACK AGP™ (100 x 4 mm; 5μm) column with silica guard column was used. The detector was set at 215 nm. The analysis was carried out at 25 °C and the injection volume was 80 μL.

Linearity of the method of quantification for MTD from 4.90 to 108 ng/mL and from 19.0 to 3280 ng/mL for plasma and urine samples, respectively was proven. The method for EDDP in urine was linear between 52.3 and 4200 ng/mL. For concentrations located at the lower, middle, and higher portions of the calibration curve, intra and inter-day coefficients of variation (precision) and relative errors (accuracy) were below 14%.

2.3. Pharmacokinetic and statistical analysis

Once concentration data were obtained for the 12 volunteers after the administration of MTD, mean concentration-time profiles of MTD enantiomers in plasma were constructed. For urinary samples of MTD and EDDP, each concentration was transformed into excretion rates, multiplying it by the volume of urine and dividing by the respective time interval. After this, each excretion rate was matched with the middle of its respective time interval.

The following pharmacokinetic parameters were calculated for both sexes: time to peak plasma concentration (T_{MAX}), maximum plasma concentration (C_{MAX}), area under the plasma concentration-time curve (AUC) from zero to the last quantifiable concentration and to infinite, plasma half-life (t½), and urinary drug recovery (U) as MTD and EDDP.

R-to-S MTD plasma concentration ratios were followed throughout time in order to seek information about enantiomers' relative clearances. Similarly, R-to-S urinary excretion rate ratios for MTD were followed throughout time in order to go deeper into their relative clearances, since data coming from this fluid is more related with free plasma concentration of analytes. On the other hand, R-to-S excretion rate ratios for EDDP give specificity for the stereoselective MTD-to-EDDP route of elimination.

Statistical significances between means were assessed by a non-paired (between sexes) and a paired (between enantiomers) Student's t-test.

3. RESULTS

Figures 1-2 show mean plasma and urinary R- and S-enantiomer of MTD exposures after the administration of 10 mg of MTD in women and in men, respectively. EDDP exposure in urine is shown in Figure 3. After 24 hours, most of the individuals showed MTD levels below the lower limit of quantifications. Then, analyte exposures were just studied within the first 24 hours after dose. Women experienced much more side effects than men did mainly with regard to nausea.

Tables 1 and 2 summarize the results found in this work, for both sexes. Both in men and in women R-MTD showed lower plasma exposure ($p < 0.001$) than S-MTD (Figure 1). However, as shown in Figure 2, urinary exposure showed an inverse relationship between isomers ($p < 0.01$).

4. DISCUSSION

Sex-differences in opioid pharmacokinetics might affect safety and efficacy of treatments, depending on the sex of patients [24]. Pharmacological activity of R-MTD would be better predicted from free plasma concentrations than from the total ones.

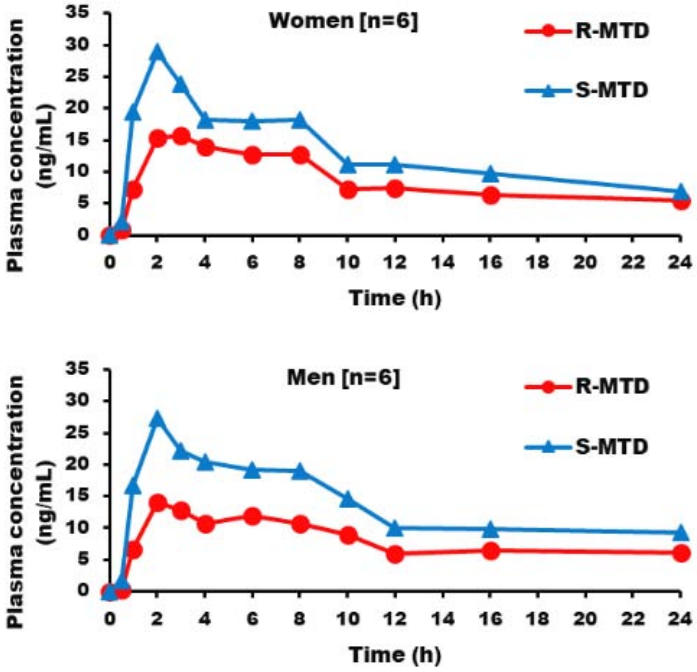

Figure 1. Mean R- and S-MTD plasma concentration-time profiles after oral administration of 10 mg of MTD in women and in men.

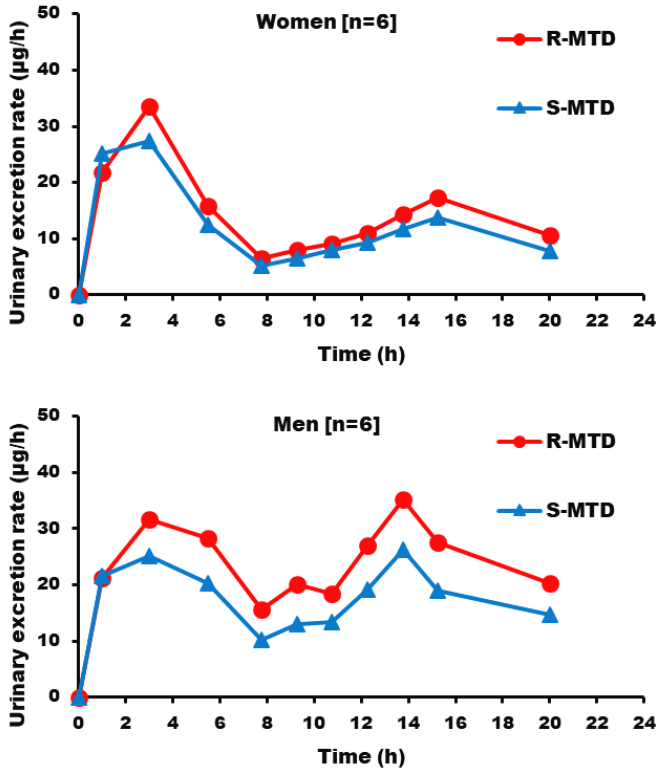

Figure 2. Mean urinary excretion rates of R- and S-MTD throughout time after administration of 10 mg of MTD in women and in men.

Table 1. Mean (± standard deviation) pharmacokinetic parameters obtained in women and in men, for MTD enantiomers in plasma after oral administration of 10 mg of MTD.

		$t_{1/2}$ ± SD (h)		AUC (0-24) ± SD (ng*h/mL)		AUC (0-inf) ± SD (ng*h/mL)		C_{MAX} ± SD (ng/mL)		T_{MAX} (range)[a] (h)	
		R	S	R	S	R	S	R	S	R	S
PLASM	Women	27.3 ±12.6	22.1 ±7.8	190[b] ±61	302[b] ±89	327[c] ±213	502[c] ±295	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	374[c] ±87	579[c] ±138	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.
[c]: $p < 0.05$, paired t-student test between R and S.

Table 2. Mean (± standard deviation) MTD and EDDP enantiomer amounts recovered in urine for women and men 24 h after an oral administration of 10 mg of MTD.

	U (0-24) * ± SD (µg)			
	MTD		EDDP	
	R	S	R	S
Women	312 ±175	270 ±166	248 ±88	463 ±203
Men	472 ±122	340 ±178	310 ±136	606 ±272

*: $p<0.01$, paired t-student test between R and S.

The higher R-MTD urinary exposure that women had (Figure 2) was well correlated with their more intense side effects presented in relation with men, mainly around T_{MAX}.

Regarding plasma results, the higher plasma protein binding of S-MTD conditioned its higher exposure. Free drug plasma exposure would be more reliable in order to assess relative elimination between isomers. Both men and women showed secondary peaks in plasma concentration profiles, revealing that MTD would follow blood-GIT-blood recirculation, as was expected [8, 9].

Regarding the excretion rate of the drug, although factors like Pgp affinity and urinary pH could affect drug passage from blood to urine, urinary MTD concentration might surrogate its free plasma levels. No stereoselectivity was previously reported for renal MTD clearance, at least following single dose administration. Then, no transporters induction can be envisaged [5] and thereafter, only pH-partition could have influenced drug passage from blood to urine. It can be said that the higher level of R-MTD excretion rate ($p < 0.01$), in comparison with S-MTD, evidences its lower intrinsic clearance, and then, an intrinsic stereoselective biotransformation in favour of S-MTD can be assessed. Although S-EDDP showed a higher ($p < 0.001$) urine excretion rate (Figure 3), no conclusions about its bioavailability could be inferred due to lack of information about the stereoselectivity of the metabolite clearance.

MTD urinary exposure did not show the same pattern of plasma secondary peaks. A deep fall in the urinary profile after lunch was observed instead. This was because urinary pH increased after food intake (urinary postprandial alkaline tide [25]), and hence, the excretion rate of MTD diminished.

Excretion rate profiles showed a constancy in the R-to-S MTD ratio once the absorption finished. This suggests that the difference between free isomer concentrations might be more related with their difference in absorption and/or fast disposition than in slow disposition (Figure 4). As it is shown in table 1, their half-lives were not different, explaining the constancy for the R-to-S ratio. Interestingly, R-to-S EDDP ratios showed practically constant values from the beginning of the experiment, except after food intake. This fact would reveal different behaviours in the systemic EDDP's formation. Due to the small amount of EDDP systemically present (around one tenth of MTD dose [26]), some stereoselective difference in MTD biotransformation might affect the R- and S-metabolite balance significantly. The highest imbalance could be noticed between 3 and 7 h post-lunch, when blood-GIT-blood recirculation

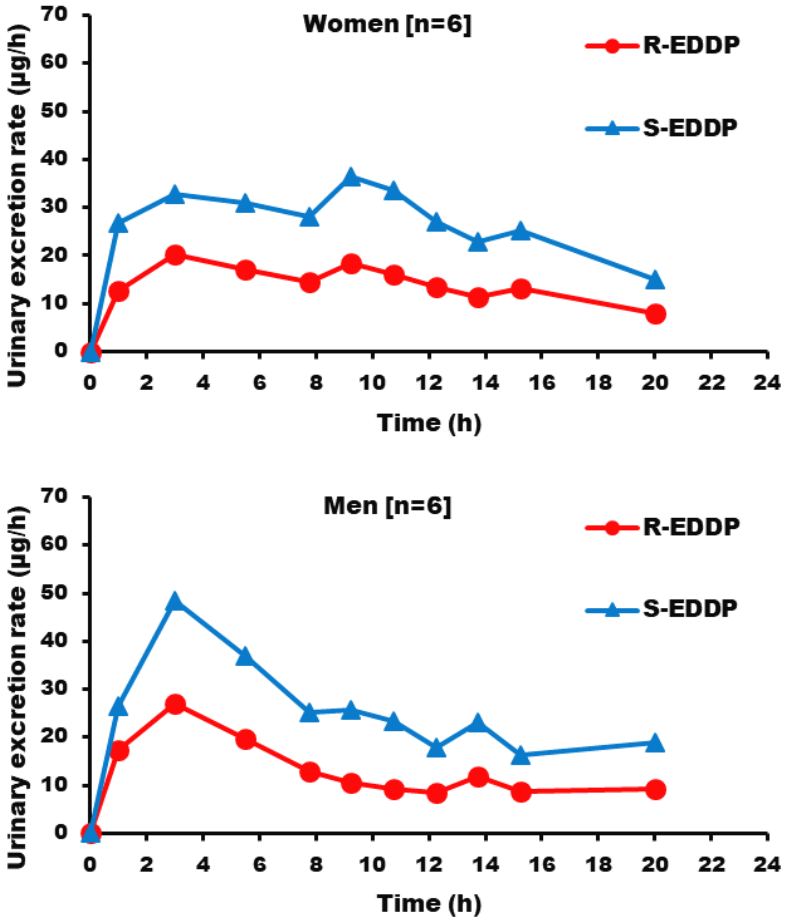

Figure 3. Mean urinary excretion rates of R- and S-MTD metabolite throughout time after administration of 10 mg of MTD in women and in men.

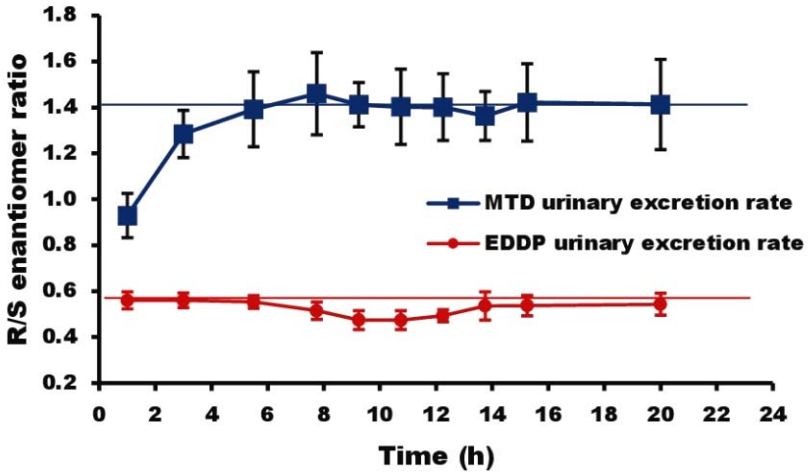

Figure 4. Mean (± 95%CI) R-to-S MTD and EDDP urinary excretion rate ratios after oral administration of 10 mg of MTD in 12 subjects. A significant decrease of the EDDP enantiomer ratio ($p < 0.05$) with respect to a basal value of 0.55 can be seen between 8 to 12 h post-dose.

of MTD would have been taking place as a consequence of gastric secretion followed by intestinal reabsorption. This cycling might not change the R-to-S MTD ratio since only free MTD flowing throughout bloodstream follows such a cycle. The minor free fraction of its systemic amount is unable to disturb the balance between total R- and S- MTD enantiomers in the body.

As mentioned in the introduction section, MTD molecules present in bloodstream go through intestinal and hepatic stereoselective metabolism through enzymes such as CYP2C19 and CYP2B6. When a re-entry process following a meal intake takes place, molecules previously secreted to gastric juice are thereafter reabsorbed through the intestine. Consequently, more MTD molecules go through the enterocyte. Our results showed that when this happened, R-to-S EDDP ratios changed. This evidenced a difference in the enantiomer metabolism stereoselectiveness between enterocytes and hepatocytes, due to a plausible difference between their enzyme relative contents. In our case, S-MTD metabolism seemed to be favoured due to the passage of MTD through the intestine to a higher extent when compared to the liver, in relation with the basal distribution between these two organs. We should bear in mind that during food digestion an increased blood flow distribution to the splanchnic zone takes place, then, the liver and all the other organs of this region receive more molecules from those flowing through the non-splanchnic region of the circulatory system. However, for drugs secreted into the gastric juice, the fraction received by the intestine is even higher since a supplementary amount of molecules, firstly destined to the stomach and thereafter transported to the lumen because of an induced gastric secretion, reaches the enterocytes.

The higher production of S-EDDP in relation with R-EDDP might be more pronounced in men because of their higher gastric secretion. However, the observed R-to-S ratios in favour of this presumption did not reach significance, probably because of the low number of subjects.

Our findings do not contradict the reported lack of stereoselectivity in bioavailability after an oral single dose of racemic MTD in healthy volunteers [20]. During the first entrance of the dose, from the formulation, the amount of drug present in the gut is high enough to saturate both stereoisomers' presystemic biotransformation. Therefore, at this time, first order MTD absorption predominates over its presystemic loss, explaining both the high and the non-stereoselective oral bioavailability [1].

5. CONCLUSIONS

These findings evidenced not only that the intestinal metabolism of MTD is relevant, but also stereoselective and different from the hepatic one. Sometimes, blood-GIT-blood recycling extends the residence of a drug in the body, but according to our results, S-MTD might not be favoured from such recycling, because every time it passes through the enterocytes its elimination increases.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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