

EDDP METABOLITE AS BIOMARKER FOR MONITORING OF METHADONE SUBSTITUTION TREATMENT

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Abstract

Currently, the surveillance of methadone substitution treatment is considered an ongoing challenge, given the need for the individualization of therapy and for increasing its efficiency. At present, although 2-ethyliden-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) is an inactive metabolite and consequently, it is assumed that the evaluation of its levels in biological samples has no utility, there are discussions on the need for such determinations; thus, quantifying the urinary EDDP metabolite levels could be useful to eliminate the false positive results of screening tests and to detect the possible manipulation of urine samples by the patients. This study aimed to quantify methadone and its main metabolite EDDP, in the patients' urine, during methadone substitution treatment. The study group consisted of long-term heroin addicts, who were under substitution therapy with methadone, with an average daily dose of about 72 mg. A liquid-liquid extraction procedure (with hexane:isopropanol 97:3) of the urine samples and a GC-MS method for methadone and EDDP quantification were applied. The results revealed a ratio between methadone and EDDP concentration supra unitary, showing a wide distribution of both substances. This indicated a slow biotransformation of methadone (in poor metabolizer patients), suggesting a possible liver pathology. Methadone concentrations were more variable than those of EDDP. No correlations between methadone doses and methadone or EDDP urinary levels have been shown, but a statistically significant correlation between methadone and its metabolite concentrations in urine has been depicted. These results indicate that EDDP can be used as a biomarker for the monitoring of methadone substitution treatment, using the testing of urine sample in order to check for compliance in patients stabilized with methadone.

Rezumat

În prezent, monitorizarea tratamentului de substituție cu metadonă este considerată o provocare continuă, având în vedere necesitatea individualizării tratamentului și creșterea eficienței sale. Deși 2-etiliden-1,5-dimetil-3,3-difenilpirrolidina (EDDP) este un metabolit inactiv și, în consecință, se presupune că evaluarea nivelurilor lui în probe biologice nu ar avea utilitate, necesitatea acestor determinări este unanim declarată. Astfel, cuantificarea nivelurilor metabolitului urinar principal al metadonei, EDDP-ul, poate contribui la înlăturarea rezultatelor fals pozitive ale testelor de *screening*, precum și la depistarea manipulării probelor de urină de către pacienți. Acest studiu vizează cuantificarea metadonei și a principalului său metabolit, EDDP, în urina pacienților, aflați în tratament de substituție cu metadonă. Grupul de studiu a fost alcătuit din dependenți de heroină pe termen lung, aflați în terapie de substituție cu metadonă, cu o doză medie zilnică de aproximativ 72 mg. Probele de urină au fost extrase cu un amestec hexan:izopropanol (97:3), iar pentru cuantificarea metadonei și a EDDP-ului a fost utilizată o metodă GC-MS. Rezultatele arată o distribuție largă a concentrațiilor urinare ale metadonei și EDDP-ului, și un raport între concentrațiile celor două substanțe predominant supraunitar. Acest lucru indică o biotransformare lentă a metadonei (pacienți metabolizatori lenți), ceea ce sugerează o posibilă patologie hepatică. Nu au fost demonstrate corelații între dozele de metadonă și nivelurile urinare de metadonă sau EDDP, dar a fost evidențiată o corelație semnificativă statistic între concentrațiile de metadonă și de metabolit în urină. Aceste rezultate indică faptul că EDDP poate fi folosit ca *biomarker* pentru monitorizarea tratamentului de substituție cu metadonă, în probe de urină pentru a verifica complianța pacienților stabiliți sub tratament.

Keywords: heroin addiction, substitution therapy, methadone and EDDP urine levels

Introduction

The current research for opiate addiction refers to the assessing of the prevalence and patterns use, the analytical diagnostic of drug consumption, the treatment of overdoses and the establishment of methadone substitution treatment procedures [1].

Providing methadone maintenance therapy, based on scientific evidence and supported by appropriate assessment and education, should be considered as essential treatment option, especially in communities with high prevalence of opioid dependence. The methadone treatment should be individualized

according to the patients' needs (e.g. genetic polymorphisms in methadone metabolism and transporter proteins), the dependence severity, the associated pathologies, methadone drug interactions, and poly-substance use [13]; also, the treatment should be monitored in order to reduce the possible side effects. The quantitation of methadone levels in biological samples has an important role in monitoring the substitution treatment, since numerous studies have shown a significant correlation between plasma concentrations and methadone dose. Thus, by quantifying the levels of methadone in biological samples, in correlation with information from the history and clinical examination, could be useful for the clinician, for monitoring methadone substitution treatment for opioid dependence [4, 15].

Methadone is rapidly absorbed after oral administration, and it is widely distributed in tissues. Methadone undergoes biotransformation to two pyrrolidine inactive metabolites, EDDP (2-ethyliden-1,5-dimethyl-3,3-diphenylpyrrolidine), the major urinary metabolite and EMDP (2-ethyl-5-methyl -3,3-diphenylpyrrolidine) [14].

Current data indicate the need for quantifying the levels of methadone in urine, in order to detect methadone extra-consumption as well as the treatment failure as a consequence of possible diversion of methadone on the illicit market. In addition, although EDDP is an inactive metabolite, it has been suggested that the determination of EDDP levels in urine would be useful to eliminate the positive results of screening tests and to detect the sample handling.

Several recent studies indicate that metabolites analysis can be useful in toxicological determinations. For instance, the detection of zolpidem phenyl-4-carboxylic acid, the main pharmacologically inactive metabolite of zolpidem would have utility in clinical and forensic settings [10]. Determination of cotinine, the main metabolite of nicotine, in biological samples can be used as a quantification marker of nicotine addiction, as well as for passive smoking [19].

Although during the procedures for urine samples collection from drug users, all measures to ensure the integrity of samples are taken, some patients show considerable ingenuity in their efforts to manipulate the samples. Possible diversion methods include sample handling, excessive water consumption, ingestion of diuretics such as herbal teas, as well as substitution of urine from another person [7, 12, 17]. National official reports showed that detainees reported higher rates of drug use and more harmful consumption patterns than the general population. Drug services offered in European prisons comprise a series of interventions, including providing information, counselling and treatment, and harm reduction measures [2]. The analysis of urine

samples is regularly undertaken for people deprived of freedom who have a history of drug use or undergo the treatment for drug addiction in detention.

Current data is a convincing argument for the usefulness of quantifying the levels of EDDP in urine, as an objective tool for monitoring methadone substitution treatment and for assessing the treatment compliance [5, 12].

In this context, the study aimed to quantify methadone and its major metabolite EDDP, in the urine of patients in detainees and to assess the possible correlation between their urinary concentrations and other parameters, such as methadone dose.

Materials and Methods

Study group

The study group consisted of 55 addicts, hospitalized in a Penitentiary hospital from Bucharest and voluntarily enrolled in the methadone substitution treatment. The study was conducted in the period August 2013 - May 2014. All patients were diagnosed with addiction to heroin, according to ICD-10. The study was approved by the Ethical Committee of the Hospital and was carried out in accordance with the Declaration of Helsinki [3]. The informed consent for the participation to the study was obtained from all patients.

Socio-demographic data, history of drug abuse, treatment history and clinical characteristics were collected from the medical records.

Biological samples

Urine samples were collected before the daily methadone dose administration, approximately 24 hours after the previous intake of methadone dose. The sample collecting was performed according to the protocol for the determination of creatinine in urine. Creatinine was determined by an enzymatic method, using commercial kits, in all samples of urine, in order to correct the concentration values of methadone and its metabolite, as literature data suggested [11, 16]. In addition, the urinary pH was evaluated, according to the literature data about its influence on methadone concentrations [8].

Sample extraction

A liquid-liquid extraction procedure was performed, using hexane:2-propanol 97:3, at basic pH [18]. Briefly, to 1 mL urine sample, 200 μ L of 2 M kalium hydroxide and 4 mL of mixture n-hexane:2-propanol (97:3) were added. The extraction was performed in a shaker for 15 min., then the samples were centrifuged 10 min at 3400 rpm at 15°C; the organic layer was then transferred into a separate tube and evaporated under nitrogen stream at 40°C. The residue was reconstituted in 100 μ L methanol and 1 μ L sample was analysed by GC-MS.

Quantitative analysis

A GC-MS method was applied for methadone and EDDP quantification in urine [6, 18]. The analysis was performed on a Focus DSQ II GC-MS, and the separation was achieved on TR-5MS capillary column (15 m × 0.25 mm I.D., 0.25 µm film thickness). The injection port (split mode) was set to 220°C. The column temperature had been initially held at 150°C for 1 min; later, it was increased to 220°C (10°C/min), then it was increased to 280°C (30°C/min) then held at 280°C for 1 min. The carrier gas was helium at 1 mL/min.

Statistical analysis. Descriptive statistics, was performed with SPSS Statistics ver. 21; data for different parameters are presented as mean ± standard deviation (SD). The correlations between the investigated parameters, using correlation coefficients (Pearson), were also evaluated. All

tests were statistically significant at threshold $p < 0.05$ and $p < 0.1$.

Results and Discussion*Characterization of the study group*

The detailed characterization of the subjects included demographic characteristics, history of drug use, consumption type, multiple drugs use and addiction treatment history.

It can be observed the prevalence of males in the study group (85% men; the male/female ratio is 5.87 (Table I). The average age was 32.56 (± 7.32) years, ranging between 17 - 55 years. Patient distribution by age group indicated the most common age groups 26 - 30 years (34.54%) and 31 - 35 years (29.10%). Most patients were long term heroin addicts; they have been used heroin for approximately 12 years (mean 11.98 years, range 1 - 24).

Table I

Demographic characteristics, history of drug use and treatment for the study group

Total cases	55
Gender distribution	47 men, 8 women
Men/women ratio	5.87
Mean age (years) (± standard deviation)	32.56 (± 7.32) (range 17 - 55 years)
Frequent age groups	< 20 years (3.64%, 2/55); 20 - 25 years (5.45%, 3/55); 26 - 30 years (34.54%, 19/55); 31 - 35 years (29.10%, 16/55); 36 - 40 years (10.9%, 6/55); > 40 years (14.54%, 8/55)
Mean age (years) at the onset of consumption	20.67 (± 8.10) (range 8 - 53 years)
Heroin use duration (years) (Mean ± standard deviation)	11.98 ± 6.00 years (range 1 - 24)
Other used drugs	Frequent consumption: methadone, benzodiazepines, cocaine, barbiturates. Occasional consumption: cannabis, Ecstasy/3,4-methylenedioxymethamphetamine (MDMA)
Methadone maintenance treatment	Yes: 56.36% (31/55) No: 43.64% (24/55)
The mean dose of methadone (mg/day) (± standard deviation)	72.08 ± 46.53 (range 10 - 190)

Quantitative analysis. Methadone and EDDP urine levels and the relationship with the methadone doses

Urine concentrations of methadone (MTD) and EDDP were determined using a modified GC-MS method [6, 18]. The changes of the method consisted in the quantification of the external standard method, the use of the mixture hexane:2-propanol as extraction solvent and the carrying out

of the calibration curves using urine as biological sample. For the identification of methadone and EDDP in urine samples, a full scan analysis technique was used. The identification was positive for both compounds, based on the retention time (Tr) (10.38 min for EDDP and 11.32 for MTD) and m/z value (72 for MTD and 276 for EDDP) and also by comparing the mass spectra with the spectra library (Figure 1 and 2).

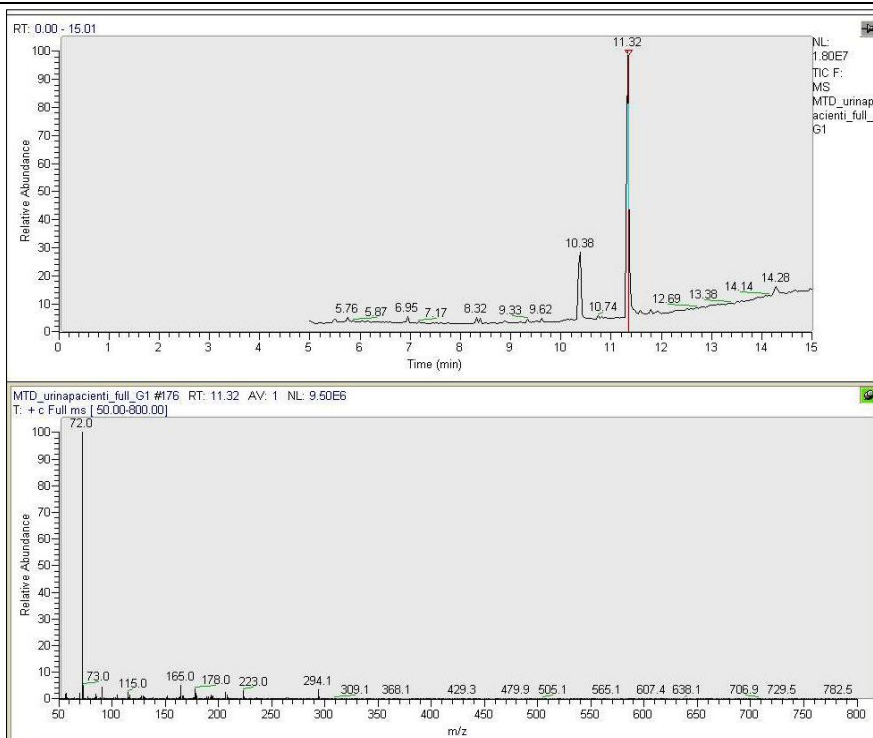


Figure 1.

Typical chromatogram and MS spectrum for MTD identification in patients' urine sample ($T_r = 11.32$ min, $m/z = 72$)

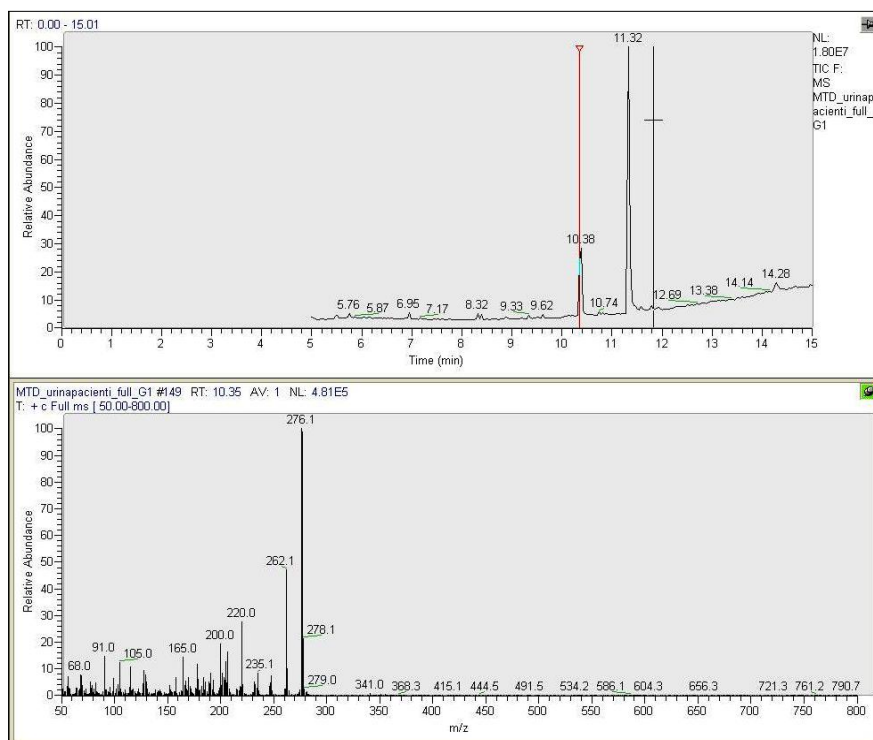


Figure 2.

Typical chromatogram and MS spectrum for EDDP identification in patients' urine sample ($T_r = 10.38$ min, $m/z = 276$)

For the quantitative determination, a secondary ion mass (SIM) analysis technique, following ions with m/z 72 (methadone) and 276 (EDDP), was used in

order to increase the sensitivity of the method. The method was tested for linearity (0.025 - 4 $\mu\text{g/mL}$, $R^2 = 0.9967$, for MTD and 0.1 - 4 $\mu\text{g/mL}$ $R^2 = 0.9944$,

for EDDP), LOD (0.008 µg/mL for MTD and 0.016 µg/mL for EDDP), selectivity, precision (RSD < 15%) and accuracy (as recovery percent after extraction: 94.37% for MTD and 92.57% for EDDP).

The GC-MS method was used for methadone and EDDP quantification in the urine samples of selected patients. A wide distribution of methadone urinary levels was registered, with a mean of 1.54 ± 1.36 µg/mL (range 0.27 - 4.93 µg/mL) (Figure 3).

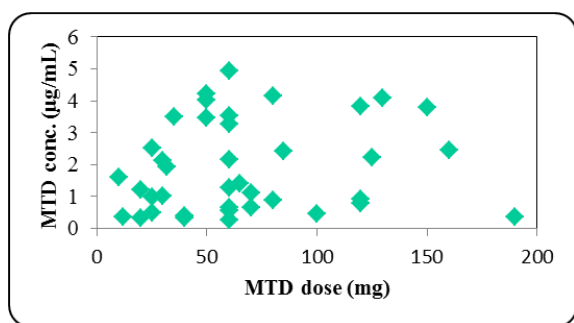


Figure 3.

The distribution of methadone urinary concentrations depending on the methadone dose

These results are consistent with other studies, indicating that urinary methadone levels in patients under methadone maintenance therapy are predominantly in the range 0.5 - 2 µg/mL [4, 5, 17]. In our study, most patients registered values of methadone concentrations below 1 µg/mL. The average of daily methadone dose administered was 72 mg (± 46.5). For the EDDP urinary levels, an average of 0.28 ± 0.615 µg/mL (range 0.100 - 3.31 µg/mL) was obtained (Figure 4).

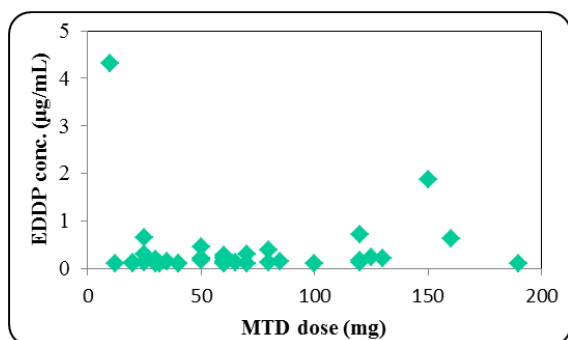


Figure 4.

The distribution of EDDP urinary concentrations depending on the methadone dose

For most patients, the values of EDDP concentrations were below 0.5 µg/mL. The results indicate that methadone concentrations were more variable than those of EDDP. The ratio between methadone urinary concentration and EDDP urinary concentration was predominantly higher than one (average 8.27 ± 6.49 , range from 0.37 to

25.62), with the highest share for the patients with values between 1 and 10 (Figure 5). This indicates a slow biotransformation of methadone (poor metabolizers patients), suggesting possible liver pathology. We underline that among chronic heroin users, usually increased rates of virus B and C hepatitis (predominantly) infection are recorded. This can lead to liver damage [15].

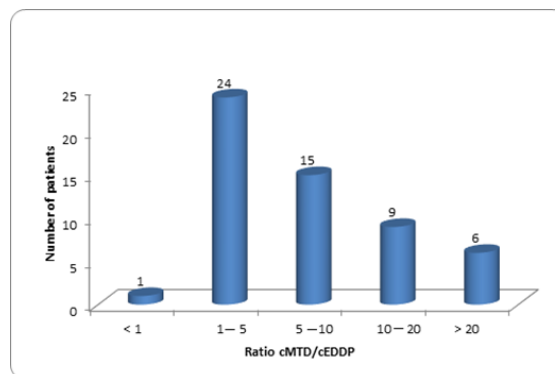


Figure 5.

The patients' distribution according to the ratio of methadone and EDDP urinary concentrations

No statistically significant correlations between methadone doses and methadone or EDDP urinary levels had been shown, suggesting the high individual variability in the pharmacokinetics of methadone, particularly regarding the metabolism and the drug elimination phase in the study group. Similar results had been obtained when the concentrations of methadone and its metabolite were corrected to the urinary creatinine values. These results are in contrast with the retrieved studies that found a regression model using urine EDDP/creatinine ratios to predict the used dose of methadone across the clinically significant range for either pain management or drug addiction [12, 17]. However, in a pilot study that investigated the relationship between administered daily methadone dose and subsequent urinary concentrations of methadone and EDDP, a large variation in urinary concentrations of methadone and its metabolite had been shown [9]. The results of the study suggested that the urinary methadone and EDDP excretion patterns could be used to monitor the individual compliance to methadone substitution treatment. This approach would be successful through long-term monitoring of individual and based only on the quantitation of every specimen received for each subject [9].

The average urinary creatinine concentration was 131.60 mg/dL (± 88.68), ranging between 18 and 511 mg/dL. Urinary pH ranged between 6 and 8 and there was no evidence of their impact on urinary concentrations of methadone metabolite (no statistically significant differences). These results are in contrast with other recent studies indicating

that urinary methadone excretion was significantly affected by pH, in which the ratio of methadone and EDDP was two times higher in acidic urine [8]. The study has revealed a high statistically significant correlation between the methadone concentrations and the metabolite concentrations in urine (Pearson $r = 0.695$, $p < 0.001$), as well as these concentrations were adjusted to creatinine levels. Recent findings showed that methadone-to-EDDP ratio in urine was consistent at 24 and 4 h, hence suggesting that outpatients have the possibility of being monitored by single urine sample in order to check for compliance [8].

The results of this study further enhance the guidelines for monitoring the methadone treatment by using the analytical determination of EDDP metabolite in urine. This is based on the assumption that in urine samples with positive test for methadone, but negative for EDDP, it is possible that the parent compound was added. In addition, the methadone-to-EDDP ratio in urine enables a general appraisal regarding the metabolism of methadone among heroin-dependent patients under methadone maintenance therapy.

Conclusions

The results of the study, consequent with the literature data, indicate a wide distribution of methadone and EDDP metabolite concentrations in urine. Over unity ratio of the urinary concentrations of methadone and EDDP suggests a slow metabolism of methadone in the selected patients. The EDDP metabolite can be used as a biomarker for monitoring the methadone substitution treatment using urine samples in order to check for compliance in stabilized by substitution therapy patients. The study may contribute to increasing the clinical utility of the analytical determination of methadone and EDDP in urine, in order to improve methadone-maintenance treatment and detecting drug diversion.

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