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REVIEW ARTICLE

Metabolomics of methadone: clinical and forensic toxicological implications and variability of dose response

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ABSTRACT

Methadone is a full μ-opioid receptor agonist used in the treatment of heroin addiction. It is commercialized as a racemic mixture with considerable variability in the pharmacokinetics and pharmacodynamics between individuals that can affect dose-response and toxicological profile. This review aims to discuss metabolomics of methadone, namely by presenting all major and minor metabolites and pharmacokinetic drug interactions. The main mechanism for methadone metabolism is hepatic through the cytochrome P450, specifically isoenzymes 2B6, 3A4 and 2D6. Firstly, methadone is *N*-demethylated and cyclize to form its major 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenylpyraline (EMDP) metabolites. Several alternate minor pathways have been described namely various methadol metabolites, which proved to be active.

It is expected that knowing the metabolomics of methadone may provide further insights, attempting a personalized therapy aiming to attain effective blood concentrations. The historical record is therefore especially important when investigating clinical and forensic cases related to methadone administration, since interindividual responses are known to vary considerably.

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Introduction

The term "opioids" refer to compounds of natural, semi-synthetic, synthetic and endogenous origin that interacts as agonists or antagonists with μ-, κ- or δ-opioid receptors and that are completely antagonized by naloxone (Martin, 1983). Analgesia, sedation, respiratory depression, cough reflex suppression, sweating, euphoria, dysphoria, confusional state, insomnia, agitation, fear, hallucinations, drowsiness, nausea, vomiting, motor incoordination, mood changes, miosis, dependence and addition are the main pharmacological effects (Dinis-Oliveira, 2014a; Katzung et al., 2012). Methadone, buprenorphine and naltrexone have been claimed useful as treatment strategies for opioid addiction (Brunton et al., 2011).

Methadone (Dolophine®), firstly developed during World War II, has been used since 1965 for the treatment of opioid addiction, typically in patients with polydrug abuse or significant psychiatric comorbidity, and as analgesic (Dole & Nyswander, 1965; Mattick et al., 2009). In comparison with other opioids, it possesses a long half-life, a property that makes outpatient management feasible (Kristensen et al., 1996; Leonard, 2003).

Although methadone therapy is generally considered safe, several risk intoxication factors have been identified, such as i) the concomitant administration of other drugs; ii) the elevated risk of some individuals for *torsade de pointes* (a life-threatening cardiac ventricular dysrhythmia) and iii) the inadequate or erroneous dose increase adjustment, namely, when prescribing methadone for pain. Indeed, time to develop tolerance to previous doses must be given especially during the first weeks of the treatment to avoid accumulation of toxic levels that can be easily achieved due to methadone long half-life. A linear relationship between opioid-related overdose deaths and the increase in the prescription of pain relievers have been described (Paulozzi & Ryan, 2006).

Methadone (an opioid of diphenylheptylamine class; structurally unrelated to morphine) binds primarily as full agonist to the μ-opioid receptor, but also possesses some affinity for the κ and δ receptors. In addition, it exerts non-opioid-related effects, namely, (Bush et al., 2006; Callahan et al., 2004; Eap et al., 2007; Johnson, 2011; Mendlik & Uritsky, 2015; Mitchell et al., 2004;

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Robinson et al., 1997) i) noncompetitive antagonist at the *N*-methyl-D-aspartate receptor that may have a role in the treatment of neuropathic pain and hyperalgesia and may retard the development of opioid tolerance; and ii) as inhibitor of the reuptake of noradrenalin and serotonin that may result in an antidepressant effect and predispose to serotonin syndrome.

As shown in Figure 1, methadone (6-dimethylamino-4,4-diphenyl-3-heptanone) has one stereogenic center and therefore can exist as two possible stereoisomers [*R* (L-methadone) and *S* (D-methadone)]. The opioid activity of methadone lies principally in the *R* enantiomer that has a 10-fold higher affinity for the μ and δ receptors and longer half-life (53 versus 33 h) (Kristensen et al., 1996; Leonard, 2003). Moreover, *R*-methadone is also approximately 50 times more potent as analgesic and the unique effective enantiomer in preventing opioid withdrawal (Leonard, 2003; Scott et al., 1948). On the other hand, *S*-methadone has been implicated in important non-opioid-related adverse effects. It blocks the rapidly activating delayed rectifier cardiac potassium channel, I_{Kr} (hERG or Kv11.1 channels encoded by the *human ether-à-go-go-related gene* or *KCNH2*) greater than twofold in comparison to *R*-methadone, being primarily responsible for cardiotoxicity (Inturrisi, 2005) and even death (Eap et al., 2007). Indeed, not all methadone-related deaths were due to respiratory depression (Kristensen et al., 1995). S-methadone also retains certain pharmacological effect such as the antitussive activity (Ferrari et al., 2004). Both enantiomers seems to be an effective *N*-methyl-D-aspartate receptor. In spite of this dualistic activity, methadone hydrochloride is most commonly administered as a 50/50 ratio of the *R/S*-methadone enantiomers since it is expensive to make it pure (Ferrari et al., 2004). The only exception is in Germany where it is also available as the pure *R* enantiomer.

Metabolomics (also known as metabolic profiling or metabonomics) aims the characterization of all picture of drug's metabolites (Dinis-Oliveira, 2014b; Dinis-Oliveira, 2016b). The focus of this manuscript is the methadone metabolism. Population studies consistently demonstrate extreme interindividual variation and unpredictability in pharmacokinetics and pharmacodynamics. Indeed, unintentional deaths are much more common after methadone administration than in any other opioid (Trescot et al., 2008). Moreover, metabolic substrates and/or inhibitors or inducers of the same CYP isoforms implicated in methadone metabolism are administered concurrently; consequently clinically significant changes in methadone concentration can occur (Ferrari et al., 2004). This work aims to review the metabolism of methadone focusing on all the major and minor metabolites and their pharmacological and toxicological effects.

Methodology

An English exhaustive literature search was carried out as previously described (Dinis-Oliveira, 2016a). Briefly, methadone and related known metabolizing enzymes and metabolites, and studies addressing drug-interactions were searched in PubMed (US National Library of Medicine) without a limiting period. Furthermore, the retrieved journal articles as well as books on methadone were reviewed for possible additional publications related to human and nonhuman *in vivo*, *in vitro* and *ex vivo* studies.

Absorption, distribuition and excretion

Methadone is typically administered *per os*. It is readily absorbed from the gastrointestinal tract with a peak plasmatic concentrations occurring after 2.5–4.4 h (Eap et al., 2002; Meresaar et al., 1981; Vos et al., 1995). Oral bioavailability is usually high (80–90%) pointing to a little first-pass metabolism (Bart & Walsh, 2013) but there is a significant variability between patients (41–95%) (Meresaar et al., 1981; Nilsson et al., 1982a). Thus, following the administration of equal doses, different blood concentrations are usually obtained, meaning that the selection of initial and maintenance doses should be carefully defined (Vos et al., 1995; Ward et al., 2012).

Due to its high lipid solubility, 98% of the absorbed methadone is rapidly distributed to the liver, kidneys, lungs and, in small proportion to the brain, here most of its pharmacodynamic effects are mediated (Dole & Kreek, 1973); 1–2% remains in the blood compartment (Meresaar et al., 1981), 60–90% bound to plasma proteins, mostly the acid α_1-globulins such as α_1-acid glycoprotein (AGP or AAG; also known as orosomucoid – ORM – glycoprotein) (Eap et al., 1990a; Garrido et al., 2000), and only in part (13.4–17.4%) to the γ-globulins (Olsen, 1973). AGP is synthesized primarily in hepatocytes and has a normal plasma concentration between 0.6 and 1.2 mg/mL (1–3% plasma protein) (Colombo et al., 2006). It acts as a carrier of basic and neutrally charged lipophilic compounds (whereas albumin carries acidic drugs) such as methadone and other opioids. The extent of protein binding is of obvious importance for methadone activity. Indeed, since α_1-acid glycoprotein is an acute phase protein, their blood concentrations increase in stress conditions, cancer and in heroin addicts (Olsen, 1973). As a consequence, there is an increase of the amount of protein-bound methadone and a decrease of free and active methadone fraction

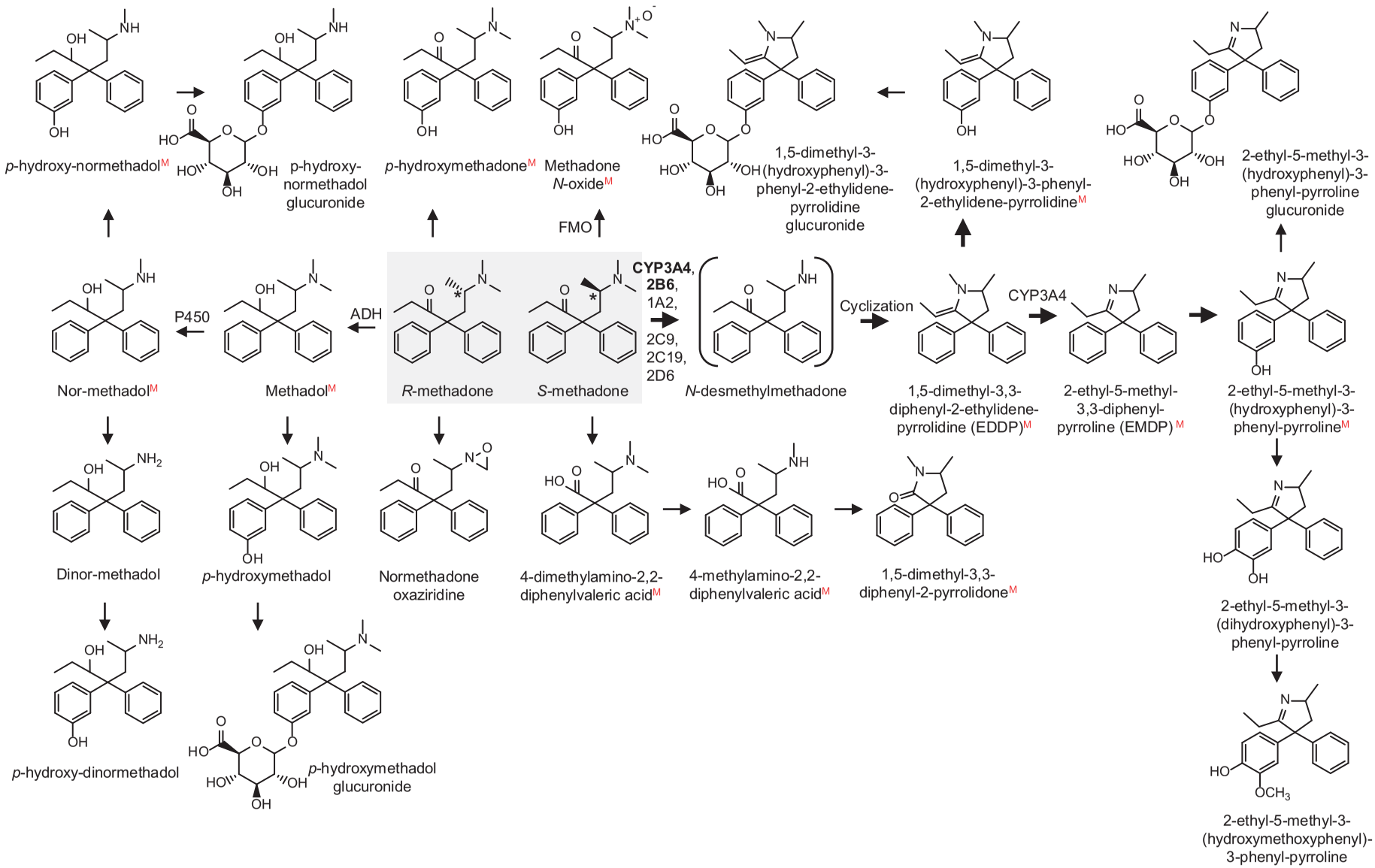

Figure 1. Metabolomics of methadone. *Indicates chiral carbon atom. Bold arrow indicates the most relevant route. ADH: alcohol dehydrogenase; FMO: flavin-containing monooxygenase; [M]metabolites already found in humans.

(Garrido et al., 1996). On the other hand, liver disease (e.g. cirrhosis) as a consequence of alcoholism may predispose to increase methadone levels and therefore to adverse effects (Arima et al., 1977).

Methadone has a large volume of distribution (2.1–9.2 L/kg) and differences have been described for *R/S* enantiomers (Dole & Kreek, 1973; Meresaar et al., 1981). Therefore, due to tissue accumulation, during maintenance treatment a short-lasting decrease of blood levels of methadone is usually not associated with clinically evident withdrawal symptoms (Plummer et al., 1988).

The elimination of methadone and its metabolites occurs mainly through the kidneys as unchanged drug, and as 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP) (Bart & Walsh, 2013; Nilsson et al., 1982a). Therefore, urine is routinely analyzed in clinical and forensic settings, namely for screening analysis by immunoassays (Dinis-Oliveira et al., 2010; Dinis-Oliveira & Magalhaes, 2013). The rate of elimination of unmodified methadone increases by urine acidification since it limits the extent of the pH-dependent tubular reabsorption (Bellward et al., 1977; Nilsson et al., 1982b). Biliary excretion has also been described (Lynn et al., 1976).

The two enantiomers differ significantly (Totah et al., 2007) in their disposition in humans, with *R*-methadone having a greater volume of distribution (3.8 versus 4.0) (Kristensen et al., 1996), longer half-life (53 versus 33 h) (Kristensen et al., 1996), higher total systemic clearance (Kristensen et al., 1996) and lower plasma protein binding (Eap et al., 1990b; Romach et al., 1981).

Metabolism

The metabolism of methadone has been described as extensive and stereoselective and occurs mainly in the liver and small intestine (Nilsson et al., 1982a). It is a very good example of how CYP isoforms can carry out a simple operation on a compound that causes a marked change in the structure and subsequent loss of function. At least 20 metabolites were already identified (Figure 1). The primary pathway of the two enantiomers is a two sequential *N*-demethylation catalyzed mainly by CYP3A4 (63–74%; not stereoselective) (Ferrari et al., 2004; Moody et al., 1997). The first methyl group is removed from the tertiary nitrogen and the resulting molecule (i.e. *N*-desmethylmethadone) is very unstable and essentially reacts with itself and immediately cyclizes by condensation of the secondary amine with the carbonyl group forming EDDP, which is then demethylated again and dehydrated to form EMDP (Sullivan & Due, 1973). Both the metabolites are inactive at opioid receptors (Pohland et al., 1971; Sullivan & Due, 1973). Nevertheless, it was demonstrated that these metabolites are likewise highly effective blockers of human $\alpha3\beta4$ and $\alpha4\beta2$ neuronal nicotinic acetylcholine receptors (nAChRs) (Xiao et al., 2001). EMDP, but not EDDP, displayed robust effects predictive of anxiolytic and antidepressant efficacy without significant effects on locomotor activity (Forcelli et al., 2016).

A number of other P450 enzymes may be important, namely CYP2B6 (12–32%), 2C19 (1.4–14%), 2C8, 2C9, 3A5, 3A7 and 2D6 (Chang et al., 2011; Crettol et al., 2005; Gerber et al., 2004). CYP2C19 ($R > S$), 2C8 ($R > S$), 3A7 ($R > S$), 2C18 ($S > R$), 2D6 ($S > R$) and CYP2B6 ($S > R$) exhibit stereoselective metabolism so that there can be significant differences in the plasma concentrations of *R* and *S* methadone even though it is administered as a 50/50 ratio (Anzenbacher & Zanger, 2012; Chang et al., 2011; Gerber et al., 2004; Totah et al., 2007). Hydroxylated metabolites of EMDP and EDDP, and hydroxy methoxy EMDP were found as *O*-glucuronide conjugates in rat bile (Lynn et al., 1976).

A minor oxidative pathway of methadone originates from 4-methylamino-2,2-diphenylvaleric acid and subsequent *N*-demethylation allowed ring closure to an additional minor cyclic metabolite, 1,5-dimethyl-3,3-diphenyl-2-pyrrolidone, which was identified in urine (Sullivan & Due, 1973). The later was reported as that possessed pharmacological activity although details are not clear (Kreek et al., 1976). *p*-Hydroxylation of one aromatic ring has also been reported, resulting in the formation of four diastereomeric compounds collectively referred to as *p*-hydroxy methadone (Anggård et al., 1975). Ketone reduction to methadol and subsequent *N*-demethylation to normethadol and dinortmethdol was described (Sullivan et al., 1972; Sullivan et al., 1973). From these only normethadol has yet been identified in humans, but all three proved to as active as methadone but are formed in low concentrations. The existence of methadone-*N*-oxide has been demonstrated (Davis & Fenimore, 1977) but conflicting results exists (Sullivan et al., 1973).

It contradicts the effective contribution of CYP3A4 and CYP2B6, regarding which one plays the major role in methadone metabolism (Totah et al., 2008). Due to its location, CYP3A4 affects both the intestinal and hepatic metabolism of methadone (Oda & Kharasch, 2001). Moreover, its activity varies greatly among the individuals, from 1- to 30-fold in the liver and from 1- to 11-fold in the small intestine (Ketter et al., 1995; Paine et al., 1997). Therefore, relevant bioavailability, interindividual differences are expected (Garrido & Trocóniz, 1999). Although clinically relevant genetic polymorphism of CYP3A4 was not yet described, this enzyme can be

highly induced or inhibited (Moody, 2013). Rifampicin, barbituates, phenytoin, carbamazepine, oxcarbazepine and antiretrovirals such as nevirapine, efavirenz, amprenavir, nelfinavir and ritonavir can accelerate the metabolism, with consequent decrease in the amount of methadone available (Meemken et al., 2015). Therefore, analgesia is reduced and withdrawal may be precipitated with consequent increased risk of reverting to illicit drug use and to incur in behaviors that facilitates the transmission of infections (Gruber & McCance-Katz, 2010). CYP3A4 is inhibited by fluconazole, ketoconazole, cimetidine, norfloxacin, fluoxetine, norfluoxetine, paroxetine, fluvoxamine, ciprofloxacine, macrolide antibiotics (e.g. erythromycin, clarithromycin, troleandromycin), grapefruit juice (large amounts) and others leading to methadone accumulation predisposing to sedation, bradycardia and/or respiratory failure (Herrlin et al., 2000). Foster et al. (Foster et al., 1999) demonstrated that methadone *N*-demethylation through CYP3A4 was not stereoselective.

CYP2B6 is a minor but highly polymorphic hepatic P450 isoform that affects about 3–4% of the Caucasians (Gadel et al., 2013; Kharasch et al., 2015). Higher *S*-methadone *in vivo* and *postmortem* blood concentrations, and QT prolongation was registered in individuals who were CYP2B6*6 poor metabolizers, the most common and clinically significant variant allele (Bunten et al., 2010; Eap et al., 2007; Kharasch et al., 2015; Yang et al., 2016). These findings may help to explain *postmortem* results linking the 2B6*6 allele to methadone-associated deaths (Bunten et al., 2011) and the required lower doses of methadone in CYP2B6 poor metabolizers (Levran et al., 2013). CYP2B6 is highly affected by many of the same inhibitors and inducers of CYP3A4 (Coleman, 2005; Anzenbacher & Zanger, 2012).

Regarding the implication of CYP1A2 in methadone metabolism, available literature data are conflicting (Shiran et al., 2009). This isoenzyme is not polymorphic but its activity can vary from 1- to 40-fold between individuals and is higher in women and induced by tobacco smoke (Klaassen, 2013).

While CYP2D6 is not induced, its expression can be reduced by paroxetine, fluvoxamine (and fluoxetine to a less extent) and exhibits genetic polymorphism (Somogyi et al., 2014). CYP2D6 ultra rapid metabolizers have reported a higher level of dissatisfaction during methadone treatment (Perez de los Cobos et al., 2007). Inhibitors of CYP2D6 have been shown to increase plasma methadone concentrations (Wu et al., 1993).

CYP2C19 has also been implicated in methadone metabolism (Tsai et al., 2014). CYP2C19 comprises 16% of the CYP2C subfamily (Gerbal-Chaloin et al., 2001). Particularly interesting are the results of some studies demonstrating that CYP2C19*2 gene variant (associated with a poor metabolizer phenotype in both the Caucasian and Asian populations) produces higher plasma concentrations of EDDP than individuals carrying the wild-type gene (Carlquist et al., 2015; Wang et al., 2013). This variant was associated with a prolongation of the QT interval of the cardiac cycle and may be useful in identifying patients with increased risk (Carlquist et al., 2015). Further mechanistic studies are needed to clarify if EDDP is implicated in this adverse effect or it is a biomarker for another metabolite(s) that effectively mediates the prolongation of the QT interval.

Flavin-containing monooxygenase have been shown to metabolize tertiary amines such as methadone to form *N*-oxide (Coleman, 2005).

Cocaine also increases clearance of methadone (McCance-Katz et al., 2010). It was also demonstrated that the microsomal demethylation of methadone is inhibited by ethanol, resulting in elevated levels of methadone in the brain and the liver (Borowsky & Lieber, 1978; Kreek, 1984). Since patients in methadone therapy, often maintain ethanol abuse, and since this substance can worsen liver disease (Dinis-Oliveira et al., 2015), higher methadone concentrations are expected (Kleykamp et al., 2015). Therefore, patients in rehabilitation should be advised to the need to abstain from street drugs.

In the course of chronic methadone treatment, induction of its own metabolism by *N*-demethylation has been described (Anggård et al., 1975; Verebely et al., 1975). This effect seems to be related to induction of CYP2B6 and CYP3A4 mRNA expression (Campbell et al., 2013) and may explain the requests for higher doses by some drug addicts (Horns et al., 1975). Moreover, another *in vivo* study demonstrated that methadone is associated with inhibition of CYP2D6 and UDP-glucuronosyl transferase (UGT) 2B4 and 2B7 (Gelston et al., 2012). The inhibitory effect on UGT2B7 is shown to have an important role in opiate withdrawal symptoms, including pupil size and tremor (Tian et al., 2012).

Conclusion & future perspective

Methadone has been widely used for the first line in the replacement therapy of heroin dependence (Johnson, 2011). Useful characteristics include route of administration, large oral bioavailability and long half-life. It is claimed, that its use has been linked to a decrease of criminal activity, the costs of crime, the illicit drug use by opiate abusers, improvement social integration and employment prospects and decrease of the morbidity and mortality of opiate users. Nevertheless, methadone therapy is complicated by unpredictable 20- to 100-fold

interindividual variability due to pharmacokinetics and pharmacodynamics factors (Inturrisi et al., 1990; Faggiano et al., 2003; Kharasch et al., 2004).

In this work, metabolism of methadone was fully reviewed. It is metabolized in the liver predominately by several isoforms of cytochrome P450 by *N*-demethylation producing EDDP and EMDP, which are the main metabolites excreted in urine. Less than 5% of methadone dose is excreted in feces. In addition, minor pathways involving aromatic hydroxylation, microsomal reduction (i.e. to methadol and subsequent *N*-demethylation to nor- or dinormethadol) and conjugation have also been reported.

Besides direct/exposure metabolites, methadone can also induce biochemical alterations, which may be of major interest in understanding potential toxic effects (Deng et al., 2012; Dinis-Oliveira, 2014b; Jiang et al., 2003; Mannelli et al., 2009). Indeed, patients undergoing methadone treatment showed altered oxidation–reduction activity confirmed by higher plasma levels of α- and γ-tocopherol and increased GSH/GSSG ratio, and altered purine metabolism evidenced by increased concentration of guanine and xanthosine, with decreased guanosine, hypoxanthine and hypoxanthine/xanthine and xanthine/xanthosine ratios (Mannelli et al., 2009). Nevertheless, these represent preliminary results and further studies are needed in this field to claim them as useful biomarkers of disease or response to methadone treatment.

To better understand clinical effects, pharmacokinetics should be explored to find for active and inactive metabolites. Indeed, the metabolic profile, namely polymorphisms in genes encoding enzymes (e.g. CPY2B6) involved in methadone metabolism, may influence both drug efficacy and toxicity. Besides genetics, due to extensive metabolism by P450, important interactions may occur when methadone is taken concomitantly with other drugs. The identification of additional metabolites is also needed during drug development and for clinical and forensic toxicology, where specific metabolites are used to confirm xenobiotic exposure (Dinis-Oliveira, 2016a). Regarding methadone, further knowledge of the pharmacological profiles of the enantiomers is required for a more efficacious therapeutic drug monitoring in order to avoid adverse effects. Particularly, *S*-methadone may interfere with normal cardiac repolarization and produce QT interval prolongation due to blockade of the myocytes hERG voltage-gated potassium channels, an effect that predisposes to the development of ventricular dysrhythmias such as *torsades de pointes* (Krantz et al., 2009), syncope and sudden death (Martell et al., 2005). The identification of genetic variants or biomarkers that may help to predict electrocardiographic changes during methadone treatment would significantly improve the safety of the drug (Carlquist et al., 2015). Finally, further studies are needed to access the contribution of drug interactions and polymorphisms of α_1-acid glycoprotein, drug transporters and opioid receptors for methadone pharmacodynamics (Li et al., 2008).

Disclosure statement

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