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Interindividual Variability of Methadone Response

Impact of Genetic Polymorphism

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Abstract

Methadone, an opioid analgesic, is used clinically in pain therapy as well as for substitution therapy in opioid addiction. It has a large interindividual variability in response and a narrow therapeutic index. Genetic polymorphisms in genes coding for methadone-metabolizing enzymes, transporter proteins (p-glycoprotein; P-gp), and μ-opioid receptors may explain part of the observed interindividual variation in the pharmacokinetics and pharmacodynamics of methadone. Cytochrome P450 (CYP) 3A4 and 2B6 have been identified as the main CYP isoforms involved in methadone metabolism. Methadone is a P-gp substrate, and, although there are inconsistent reports, *ABCB1* genetic polymorphisms also contribute slightly to the interindividual variability of methadone kinetics and influence dose requirements. Genetic polymorphism is the cause of high interindividual variability of methadone blood concentrations for a given dose; for example, in order to obtain methadone plasma concentrations of 250 ng/mL, doses of racemic methadone as low as 55 mg/day or as high as 921 mg/day can be required in a 70-kg patient without any co-medication.

The clinician must be aware of the pharmacokinetic properties and pharmacological interactions of methadone in order to personalize methadone administration. In the future, pharmacogenetics, at a limited level, can also be expected to facilitate individualized methadone therapy.

Methadone, a synthetic μ-opioid receptor agonist, is used in the treatment of severe, mostly subacute and chronic pain and as a maintenance treatment for opioid-dependent individuals. Numerous studies, the first of which was published in the mid-1960s, demonstrated that heroin addiction is a medically treatable disease and that methadone is an effective treatment for opioid dependence, reducing illicit drug use, risk of HIV infection, mortality, crime, and unemployment, and improving social stabilization, retention rates in treatment, and patients' contribution to society.[1-4] Methadone is cost effective for substitution therapy in

opioid dependence, with cost-benefit analysis indicating savings of $4–$5 in health and social costs for every dollar spent on methadone maintenance treatment (MMT).[5] Methadone substitution is also the most widely used treatment for opioid dependence and is sanctioned by the World Health Organization (WHO). It is estimated that about 500 000 people worldwide currently receive MMT. The methadone guidelines for prescribers and pharmacists prepared for the Public Health Division of the Australian Government provide detailed steps for MMT. A summary of these guidelines has been selected for clinicians and presented in tables I, II and III.[6]

Table I. Assessing the patient for methadone initiation treatment

Current level of drug use
All the drugs they use
The frequency of drug use
The quantity they use on a daily or weekly basis
The length of the current period of use
The date and time of their most recent drug use
Degree of neuroadaptation and dependence on opioids
Evaluate the patient's neuroadaptation to and dependence on opioids to determine appropriate treatment
History of drug use
Previous treatment for substance use
Psychiatric and medical co-morbidity
Physical and mental state
Social and personal history
Risk of methadone toxicity
Urinalysis to screen for opioids, benzodiazepines, psychostimulants, and antidepressants
Establishing suitability for treatment (WHO Dependence Criteria)
Withdrawal syndrome
Tolerance
Use of opiates to avoid/relieve withdrawal
Subjective compulsion to use
Narrowing repertoire of behavior
Placement of increasing importance on the use of opiates at the expense of other behaviors
Early relapse into opiate use following cessation
Providing patient information
The dynamics of stabilization
The hazards of polydrug use, particularly in the first week of treatment
The effects and adverse effects of methadone use
Program guidelines and conditions
The risks of driving while stabilizing
Expected behavior
Risks and symptoms of an overdose

Although the use of methadone in opioid dependence has overshadowed its use as an analgesic, there is growing interest in methadone for the treatment of cancer pain and other chronic pain syndromes, including neuropathic pain. The advantages of methadone include greater oral bioavailability and faster onset than morphine, absence of active metabolites, and *N*-methyl-D-aspartate (NMDA) receptor antagonism, have been widely recognized. Methadone is generally described as a second-line opioid when patients have not responded to or have developed intolerable adverse effects to first-line opioids such as morphine.[7-10] Methadone is administered as a racemic mixture of levo or (*R*)- and dextro or (*S*)-methadone, even though the (*R*)-enantiomer accounts for the majority, if not all, of the opioid effect.[1,11] There is a large interindividual variation of methadone blood levels for a given dose, which contributes to the interindividual variability in patients' response to treatment.[12,13] Interactions of methadone and genetic polymorphism in the genes encoding methadone-metabolizing enzymes (cytochrome P450 [CYP]), the transporter protein (p-glycoprotein; P-gp), and the receptor can explain this variability.

This review outlines the pharmacokinetic properties, drug interactions, and pharmacogenetics of methadone. It also aims to help physicians recognize the peculiarities of methadone and achieve optimum therapy for the individual patient.

1. Methadone Pharmacokinetics and Effects

Methadone is a highly lipophilic drug that is suitable for a variety of administration routes. Oral methadone is subjected to an important first-pass effect in the liver and gastrointestinal tract and is detectable in plasma within 30 minutes of administration.[14,15] Its bioavailability is 85% (range: 67–95%), which is three times that of morphine.[16] The peak plasma concentration occurs at 2.5–4.4 hours after dose intake (t_{max}), with some differences among patients (range 1–5 hours), but independently of the dose.[14,17]

Methadone is highly bound to plasma proteins, predominately to α_1-acid glycoprotein. α_1-Acid glycoprotein is an acute phase protein, and its concentration rises in stress conditions and in heroin addicts.[5] The variations in methadone binding to plasma proteins, resulting from the marked changes in α_1-acid glycoprotein concentration, may alter methadone pharmacokinetics.[18]

The elimination of methadone is mediated by biotransformation, followed by renal and fecal excretion. It has an elimination half-life of about 22 hours. Methadone is metabolized by *N*-demethylation.[19] Its main metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), is formed by *N*-demethylation followed by cyclization and is inactive.[20] EDDP undergoes further *N*-demethylation to 2-ethyl-5-methyl-3,3-diphenyl-1-pyrrolidine (EMDP). Two minor metabolites, methadol and normethadol have similar pharmacologic activity to methadone.[21] Nine meta-

Table II. Induction into methadone treatment – summary guidelines for prescribers and pharmacists

Factors determining the initial dose
The degree of neuroadaptation to opioids
Concurrent medical conditions, including impaired hepatic function
The time since the patient's last drug use
The patient's state of withdrawal or intoxication
Interactions with other prescribed medications
The perceived likelihood of the patient's misuse of alcohol, prescription drugs, or illicit drugs
The patient's weight
Timing of the first dose
If methadone is commenced in the morning and early in the week, it may facilitate review for evidence of methadone or combined drug toxicity when peak blood levels occur 2 to 4 hours after administration, before the third or fourth dose
The prescription
Indicate the initial dose, not a dose range, on the prescription. Do not increase the dose without personally reviewing the patient
Limit the prescription duration to 1 week, to encourage the patient to return for review during the first week (the highest risk period of treatment)
Do not prescribe commencement doses of above 40 mg for patients seeking treatment for dependence on prescription opioids (morphine, codeine, oxycodone, or pethidine)
Review during the first week
Review the patient's condition before the third or fourth dose. A review 2–4 hours after the last dose, when peak blood levels occur, will enhance your assessment of methadone toxicity. The review enables you to determine the most effective dose and manage the high risk of methadone or combined drug toxicity and overdose during induction into treatment

bolites, including EDDP, have been identified in urine and three metabolites in feces.[22-24]

Renal excretion is variable and pH dependent. At a urinary pH above 6, renal clearance is only 4% of the total drug elimination. When urinary pH drops below 6, the unchanged methadone excreted by the renal route is approximately 30% of the total administered dose. Despite this, methadone does not accumulate in patients with renal failure and is poorly removed by hemodialysis.[25] The renal excretion of EDDP (primary metabolite) is not pH dependent.

In addition to analgesia, methadone produces sedation, miosis, euphoria, and respiratory depression. Analgesia and typical opioid effects are the result of agonism at the μ-opioid receptor. Methadone also has non-opioid actions, including inhibition of the reuptake of monoamines (serotonin, norepinephrine) and noncompetitive antagonism at the NMDA receptor, which might contribute to its antinociceptive properties.[12] NMDA receptor antagonism may also attenuate the development of tolerance to methadone.

The adverse effects and toxicity of methadone are similar to those associated with other μ-opioid receptor agonists. These include respiratory depression, nausea, vomiting, dizziness, mental clouding, dysphoria, pruritus, constipation, increased pressure in the biliary tract, urinary retention, and hypotension.[12,26] Long-term treatment with methadone results in tolerance to its effects. Methadone is also known for the possible occurrence of QT interval prolongation, which is seen less with other opioid receptor antagonists. Of all adverse reactions, respiratory depression and prolongation of the QT interval are two important and potentially life-threatening ones.

Respiratory depression can be a serious problem for patients starting MMT because they are only partially tolerant to opioid agonist effects. The risk of overdose is high during this period because some patients are not opioid-dependent, misuse other drugs during treatment, or received an excessive dosage. It is important that these risk factors are identified when initiating MMT. In tolerant patients, after an induction period, dosage increases should be made in steps (small increments) so as to avoid overdose and respiratory depression. Full tolerance to the opioid effects of methadone may never completely develop even after long-term MMT. So, even in tolerant patients, the introduction of an inhibitor of methadone metabolism might induce respiratory depression. Recent studies[27,28] report that methadone alone or in combination with other drugs and/or with the existence of other factors (such as congenital long QT syndromes), can prolong QT the interval and result in severe, or even fatal, arrhythmias (torsade de pointes).

2. Cytochrome P450 (CYP) and Methadone Metabolism

Methadone is mainly metabolized in the liver and intestine.[29] Many studies have demonstrated that CYP3A4 and CYP2B6 are major CYP isoforms involved in methadone metabolism,[30-32]

Table III. Maintenance of methadone – summary guidelines for prescribers and pharmacists

Maintenance dose
Prescribing should not focus on reducing the dosage to a level to minimize the risk of adverse effects or decrease dependence, but rather on effectively controlling the patient's craving for and continued use of illicit opioids
The maintenance dose should be individualized to the patient's needs
Evidence indicates that a maintenance dose of at least 60 mg/day is more effective than lower doses in achieving treatment outcomes such as decreased illicit drug use
The methadone prescription
In the first 2 months of treatment while the patient is being stabilized and the risk of toxicity is high, prescribe a precise dose. Any adjustment to dose requires your review of the patient. After stabilization, prescribers may indicate a dose range of up to 10 mg (dose ± 5 mg)
To avoid misunderstanding, it is strongly recommended that the following information be included on the prescription:
the date when the first dose is to be dispensed
the date when the authorization to dispense will end (to encourage the methadone patient to attend for review at an appropriate interval)
the name of the pharmacy at which the methadone is to be dispensed
Review of patient progress
Prescribers should have regular contact with patients throughout their treatment with methadone. To ensure attendance for review, limit the life of the prescription to the period between reviews
In general, patients should be seen at least twice during the first week, and at least weekly during the initial 1-month period of stabilization. See the patient at least fortnightly during the second and third months, then maintain regular contact at least every 3 months (preferably monthly) while the patient is on the methadone program
Factors for review of treatment
The effect on illicit opioid use
The patient's use of other drugs
Change in the patient's lifestyle, social functioning and situation
The patient's physical health and wellbeing
Achievement of mutually agreed goals
Change in the patient's legal status
The regularity of attendance and administration
A psychological assessment
Other considerations (HIV, hepatitis B and C status, etc.)

with CYP2D6 being involved to a lesser extent.[32-34] Other CYPs, such as CYP1A2,[20] CYP2C8,[32] CYP2C9,[35] and CYP2C19,[31,35] could also be implicated in methadone metabolism. Interindividual variability of CYP enzyme activity accounts for a substantial portion of the interindividual variability in the clearance and plasma half-life of methadone.

2.1 CYP3A4

In vitro studies have demonstrated that CYP3A4 is the main CYP isoform involved in methadone metabolism.[36] Its involvement in methadone metabolism *in vivo* is suggested only by interaction studies with CYP3A4 inducers or inhibitors.[12] *In vitro* experiments conducted by Iribarne et al.[37] showed that methadone is extensively metabolized by CYP3A4.[37] Indeed, *N*-demethylation of methadone strongly correlated with the monooxygenase activities of CYP3A4 (i.e. estradiol-hydroxylation, testosterone β-hydroxylation, and nifedipine oxidation). In addition, methadone metabolism was inhibited by inhibitors of CYP3A4, whereas CYP2D6 and CYP2C inhibitors did not inhibit methadone metabolism.

Another study with a limited number of patients (n = 32) showed higher CYP3A4 activity (as measured by the midazolam phenotyping test) in patients receiving high methadone doses.[38] This high activity possibly contributed to the need for high doses because of an increased metabolic clearance, although auto-induction of CYP3A4 cannot be excluded.[38] The significance of the different metabolic enzymes is multifactorial: in a liver with a relatively high content of CYP2D6, the role of CYP3A4 is diminished.[39]

CYP3A4 activity varies greatly (up to 40-fold) among individuals, and may be affected by health status, environmental aspects (smoking, diet, comedication), hormonal, or genetic factors;[40,41]

most of this variability is thought to be genetically determined.[42,43] Over 30 single nucleotide polymorphisms (SNPs) of CYP3A4 have been described; most of the SNPs occur with low allelic frequencies (below 5%).[44] The first genetic CYP3A4 polymorphism described was the promoter variant allele *CYP3A4**1B, identified by association with a propensity toward or progression of prostate cancer.[30,45] The allele frequency showed a large interethnic variation: 2–9% in Caucasians, 35–67% in African Americans, and 0% in Taiwanese and Chinese subjects.[46,47] An *in vitro* study showed that the *CYP3A4**1B variant allele is associated with a 1.5-fold increase in transcription.[48] Other reports indicated no change in enzyme activity.[49] The *in vivo* study examining the genetic factors influencing methadone kinetics and response to treatment, showed that the impact of the *CYP3A4**1B variant on trough methadone plasma levels represents a 1.4-fold increase for (*S*)-methadone and 1.1-fold increase for (*R*)-methadone, which demonstrated that *CYP3A4**1B carriers have higher methadone plasma levels and need lower dose of methadone.[50] There are indications that the *CYP3A4**2 (664T>C; Ser222Pro), *17 (556T>C; Phe189Ser), *4(352A>G; Ile118Val), *5(653C>G; Pro218Arg), and *6 (831 insA; frameshift) alleles encode proteins with decreased CYP3A4 activity.[51-53] Increased CYP3A4 activity has been described for *CYP3A4**18 (878T>C; Leu293Pro).[52]

2.2 CYP2B6

In two *in vitro* studies, CYP2B6 was shown to be an important contributor to methadone metabolism, with an observed stereoselectivity toward the (*S*)-enantiomer.[31,54] In one study, the generation of EDDP from methadone was found to be due mainly to CYP2B6. Furthermore, a stereoselectivity toward the (*S*)-enantiomer has also been confirmed.[31] A second study found that human liver microsomal methadone metabolism is catalyzed by both CYP3A4 and CYP2B6.[54] *In vivo* studies also reported the implication of CYP2B6 in (*S*)-methadone metabolism.[35] In another *in vitro* study, Totah et al.[55] confirmed that *N*-demethylation by CYP2B6 was stereoselective whereas by CYP3A4 it was not. The authors concluded that CYP2B6 was also a major determinant of *in vivo* methadone metabolism, and its stereoselective metabolic interactions may lead to intra- and interindividual variability of methadone in patients.

A wide interindividual variability in the expression and activity of CYP2B6 in human livers has been reported *in vitro*, which can be explained by exposure to inducers or inhibitors and genetic polymorphism.[56-59] In the *in vivo* study it was demonstrated that *CYP2B6* genotype significantly influenced plasma levels of (*S*)-methadone and, to a lesser extent, (*R*)-methadone . Multiple SNPs within the *CYP2B6* gene, located on chromosome 19q13.2, have been described. The 1459C>T genetic polymorphism (Arg487Cys), present in *CYP2B6**5 and *7 alleles, corresponds to lower CYP2B6 protein levels in heterozygous and homozygous variant individuals when compared with *CYP2B6**1 wild types.[56]

With respect to enzymatic activity, Lamba et al.[59] showed an interesting correlation between the 1459C>T SNP (*5 and *7 alleles) and CYP2B6 activity in Caucasian females ($p = 0.0015$).[59] Other authors showed that *CYP2B6* *6/*6 homozygous individuals (Gln172His, Lys262Arg) have low CYP2B6 protein levels; the allelic frequency for *CYP2B6**6 is 26% in Caucasians and 16% in Japanese.[60,61] The encoding of decreased CYP2B6 activity by this allele is supported by the high plasma concentration or area under the concentration-time curve (AUC) of efavirenz (a CYP2B6 substrate) that has been observed in *CYP2B6* *6/*6 individuals.[62] Efavirenz studies further indicated that presence of the 516G>T SNP (encoding Glu172His, present in *6, *7, *9, and *13 alleles) was correlated with a 3-fold decrease in activity of CYP2B6 compared with *CYP2B6* *1/*1 individuals. A higher clearance of bupropion was shown for the *CYP2B6**4 (785A>G; Lys262Arg) variant allele, which demonstrated an increase in enzymatic activity in *CYP2B6**4 individuals.[63]

In a MMT study of 209 patients, steady-state trough and peak (*R*)-, (*S*)-, (*R*, *S*)-plasma levels and peak-to-trough plasma level ratios were measured. The (*S*)-methadone plasma concentration was shown to be highest for the *CYP2B6* *6/*6 genotype, which indicated that this genotype is associated with a poor metabolizer (PM) phenotype, but the influence of *CYP2B6* genotype on response to treatment has not been shown.[35] The stereoselectivity of CYP2B6 towards the inactive (*S*)-enantiomer of methadone is the probable explanation, at least in part, for the similar distribution of *CYP2B6* genotypes between responders to treatment requiring a low dose of methadone and those requiring a high dose, as well as between responders and nonresponders. On the other hand, the *CYP2B6**5 allele was not observed to have an influence the plasma levels of methadone.[35] Whole-cell patch-clamp experiments using cells expressing human-ether-a-go-go-related gene (hERG) show that (*S*)-methadone blocked the hERG current 3.5-fold more potently than (*R*)-methadone. The mean heart-rate corrected QT (QTc) was higher in *CYP2B6**6/*6 individuals than in non-*6/*6 individuals, which demonstrated that the *CYP2B6**6/*6 genotype was associated with an increased risk of prolonged QTc.[64]

2.3 CYP2D6

A few *in vitro* and *in vivo* studies showed a minor impact of CYP2D6 on methadone metabolism.[31,32,50] Observed interactions between methadone and CYP2D6 inhibitors seemed to indicate a more important involvement, with a stereoselectivity toward the (*R*)-enantiomer, possibly through a pathway other than *N*-demethylation.[12,33,34,65] However, the activity of CYP2D6 influences the pharmacokinetic and pharmacodynamic properties of methadone. The CYP2D6 enzyme is mainly expressed in the liver,

and is subject to genetic polymorphism. One hundred allelic variants of the *CYP2D6* gene have been identified;[66] of these,*3–*8 are nonfunctional, *9, *10, *41 have reduced function, and *1, *2, *35, and *41 can be duplicated, resulting in greatly increased expression of functional CYP2D6.

Allele combinations determine CYP2D6 phenotype, including poor metabolizer (PM, two nonfunctional alleles), intermediate metabolizer (IM, at least one reduced functional allele), extensive metabolizer (EM, at least one functional allele) and ultra-rapid metabolizer (UM, multiple copies of a functional allele and/or allele with promoter mutation).[67] The prevalence of the PM phenotype shows marked ethnic differences, with a mean value of 7.4% (4–10%) of the population in Europe and lower frequencies of 1% (0.6–1.5%) in Asians.[34,68] The majority of the Caucasian population is CYP2D6 EM (60–70%), and 1–10% of the Caucasian population is CYP2D6 UM.[68] There are also large interethnic differences in the frequencies and distribution of *CYP2D6* variant alleles. In general, 71% of *CYP2D6* alleles in Caucasians are functional alleles, while nonfunctional alleles represent 26%. The nonfunctional *CYP2D6**4 (allele frequency 20%) is carried by 75% of CYP2D6 PMs. In contrast, in Asians only ~50% of the *CYP2D6* alleles are functional. The reduced function allele *CYP2D6**10 has an allelic frequency of ~40% in Asians, causing a population shift towards a lower mean CYP2D6 activity. For African Americans and Africans, reduced function alleles represent 35% of *CYP2D6* genes, with *CYP2D6**17 being the main contributor.[69]

CYP2D6 genetic variability has also been reported to account for some of the interindividual variability in pharmacokinetic studies. The blood concentrations of (*R*)-, (*S*)-, and (*R*,*S*)-methadone are significantly different between UMs and PMs, with higher concentrations in PMs.[34,50] Importantly, this may have an impact on the successful treatment of opioid dependence in those individuals, as treatment success varied between 40% (UM) and 72% (PM).[34] Doses higher than 100 mg/day are required by 50% of UMs and 28% of PMs. Though the UMs had the lowest plasma concentrations of (*R*)-methadone, the miotic effects of (*R*)-methadone were not particularly small.[70] In addition, the CYP2D6 PMs did not present significantly different methadone plasma levels compared with EM or IM, possibly because of compensatory activity by other CYP isoforms or inhibition of CYP2D6 by some medication, including methadone, in CYP2D6 PMs. On the other hand, the heroin-dependent patients who are CYP2D6 UMs report deficient satisfaction with their usual methadone treatment, whereas PMs do not report dissatisfaction with their methadone treatment.[71] Male UMs (n = 7) reported lower satisfaction with MMT than female UMs (n = 4; $p < 0.022$), which supports another study reporting that males on MMT have higher CYP2D6 activity than females.[72]

2.4 CYP1A2

An *in vitro* study measuring the formation of EDDP from methadone using human liver microsomes suggested that CYP1A2 is not involved in this metabolic pathway.[36] *In vivo* CYP1A2 was not shown to be involved in methadone metabolism.[31,37] Large interindividual variation in expression of this enzyme has been observed (40-fold based on messenger RNA [mRNA]).[73] At present, 16 variant alleles have been documented, but the *CYP1A2* genotype does not appear to influence (*R*)-, (*S*)-, and (*R*,*S*)-methadone plasma levels,[50] which suggests that this isozyme does not contribute to methadone metabolism *in vivo*. However, these results should be interpreted with care because of the low number of PMs for CYP1A2.

2.5 CYP2C9 and CYP2C19

A few *in vitro* studies have shown the involvement of CYP2C9 and CYP2C19 in methadone metabolism.[20,31,36,39] In particular, one study has shown an important involvement of CYP2C19, with a stereoselectivity toward the active (*R*)-enantiomer for this isoform.[31] Large interindividual variability of the activities of CYP2C19 and CYP2C9 has also been described, with the existence of genetically determined PMs and EMs for CYP2C9 and CYP2C19.[74]

For the *CYP2C9* gene, two variant alleles (*2 [430C>T; Arg144Cys] and *3 [1075A>C; Ile359Leu]), have been shown to affect catalytic functions of the CYP2C9 enzyme.[75-77] Allele frequencies in Caucasians are approximately 82% for the wild-type *CYP2C9**1, 11% for *CYP2C9**2, and 7% for *CYP2C9**3.[78] For the *CYP2C19* gene, *CYP2C19**2 and *CYP2C19**3 are the most predominant null alleles.[79,80] The frequencies for allele *2 and allele *3 were reported as 15% and 0.04%, respectively, in Caucasian populations.[81]

Crettol et al.[35] demonstrated in *in vivo* studies that *CYP2C9* and *CYP2C19* genotypes do not influence (*R*)-, (*S*)-, or (*R*,*S*)-methadone plasma concentrations. This is in agreement with the similar distribution of *CYP2C9* and *CYP2C19* genotypes between responders and nonresponders to methadone treatment.[20,36]

Multiple CYPs involved in methadone clearance possibly provide alternative pathways that can take over the metabolism of methadone when one enzyme is functionally impaired, such that a specific genetic polymorphism in a CYP may have only minor global effects on the metabolic elimination of methadone. However, the involvement of multiple CYPs also results in an increased possibility of drug-drug interactions.

3. Drug Transporter Effects on Methadone Kinetics

Various *in vitro* and animal models have been used to demonstrate that methadone is a substrate of P-gp,[82-84] a transmembrane efflux transporter belonging to the adenosine triphosphate-binding

cassette (ABC) family, and encoded by the multidrug resistance 1 (*ABCB1*) gene. P-gp is expressed in various human tissues, including the intestines, liver, kidneys, lymphocytes, placenta, and blood-brain barrier.[85] In the gastrointestinal tract and hepatocytes, P-gp has the ability to influence the bioavailability of orally administrated substrates.[86,87] The activity in the liver, kidney, and small intestine can play an important role in the clearance of substrates.[88,89] The activity of P-gp at the blood-brain barrier is of particular importance to substrates with a CNS site of action, such as methadone. In P-gp knockout mice, the brain concentrations of both (*R*)- and (*S*)-methadone were increased, as were the antinociceptive effects of methadone, compared with wild-type mice.[84,90] In a randomized study of healthy subjects, Kharasch et al.[54] showed the role of P-gp in the intestinal absorption of methadone and the potential drug interactions with substrates of P-gp. This study supports the assertion that methadone is a substrate of human intestinal P-gp. However, in this study the role of quinidine as an inhibitor of other methadone transporters was not studied; in addition, quinidine has no effect on the brain P-gp, suggesting that quinidine is not an appropriate inhibitor to use to study the involvement of P-gp in methadone absorption.

Significant interindividual variability was observed in P-gp expression and function. For example, liver *ABCB1* mRNA expression in healthy subjects varies 200-fold, with a corresponding 20- to 50-fold variability in protein levels.[91,92] Similarly, there is a 2- to 10-fold variability in intestinal P-gp expression.[93] In addition to physiological and environmental factors, the naturally occurring genetic polymorphisms of the *ABCB1* gene are contributing to this variation. Indeed, the *ABCB1* gene encoding P-gp is highly polymorphic, with over 100 SNPs identified in both the coding and noncoding regions of the gene, with significant linkage disequilibrium.[85] Five common SNPs observed in Caucasian populations are the exon 2 (61A>G) and exon 11 (1199G>A) SNPs and the strongly linked exon 12 (1236C>T), exon 21 (2677G>T), and exon 26 (3435C>T) SNPs. Of these, the most investigated is the nonsynonymous exon 26 SNP, 3435C>T, which is observed with a frequency of 50–60% in Caucasians, 40–50% in Asians, and 10–30% in Africans.[94,95] It has been reported that the 3435C>T variant is associated with lower P-gp expression.[96,97] Furthermore, Wang and Sadee[98] also provided strong evidence that the *ABCB1* 3435T allele may alter the stability of *ABCB1* mRNA, demonstrating that this allele was associated with lower mRNA levels as the result of an effect on the mRNA secondary structure. Other studies have shown that SNP 2677G>T (exon 21) may be associated with decreased transporter function or expression.[99]

With regard to the functional consequences of the *ABCB1* genotype for methadone, *in vivo* human studies have also been conducted. In 245 patients undergoing MMT, *ABCB1* 3435C>T and 61A>G polymorphisms were found to have an influence on trough, but not peak, methadone plasma levels and a similar trend was observed for *ABCB1* 2677G>T.[50] In a study of 51 healthy volunteers receiving a single methadone dose,[70] *ABCB1* 2677G>T and 3435C>T were observed to have no influence on the methadone AUC and peak plasma levels; in addition, 2677G>T and 3435C>T SNPs did not exhibit any associations with the miotic effects of (*R*)-methadone.[70] A lack of significant association between *ABCB1* alleles (61A>G, 1199G>A, 1236C>T, 2677G>T, and 3435C>T) and daily dosage requirement has been reported in opioid-dependent individuals.[100]

Previous studies regarding *ABCB1* pharmacogenetics have yet to identify any single SNP that can sufficiently predict methadone response. Several clinical studies have been conducted to assess the influence of haplotypes formed by multiple SNPs on the clinical response to methadone. For example, the investigation of haplotypes formed by the SNPs at positions 61, 1236, 1199, 2677, and 3435 revealed that subjects homozygous for the wild-type (61A/1199G/1236C/2677G/3435C; AGCGC) haplotype required nearly 1.7- and 1.8-fold higher methadone doses than heterozygous and noncarriers, respectively. In addition, carriers of the AGCTT haplotype required 1.6-fold lower doses compared with noncarriers.[100] In MMT subjects, it has been demonstrated that the carriers of haplotype 61A/2677G/3435C (n = 170) presented a 1.2-fold increased in both (*R*)- and (*S*)-methadone plasma levels compared with noncarriers of this haplotype.[50] Conversely, Lotsch et al.[70] investigated an *ABCB1* haplotype based on the 2677 and 3435 SNPs only and found no significant relationship with the miotic effects of (*R*)-methadone in healthy subjects after a single dose of (*R*)-methadone. The discrepancies between these studies are possibly because of differences between the methadone formulations administered [racemic vs (*R*)-methadone], previous opioid exposure (opioid-dependent vs healthy subjects), or treatment duration (single dose vs maintenance treatment). In addition, it is possible that the miotic response to methadone as an endpoint may not be sufficiently sensitive to detect differences between haplotype groups.

However, it is clear that consideration of the effect of *ABCB1* haplotypes, instead of individual SNPs, is more likely to accurately predict P-gp expression and function. It is also evident that P-gp can transport a wide range of substrates and can be inhibited and induced.[85] Ethnic differences in the effects of *ABCB1* SNPs and haplotypes have also been observed,[95,99,101] with Caucasian Americans having a higher frequency of enhanced *in vivo* P-gp expression compared with African Americans. Accordingly, ethnicity and prior drug exposure need to be considered when analyzing the effects of *ABCB1* genetic variability on the effects of methadone in individual patients.

4. Effects of the μ-Opioid Receptor

Interindividual variability is observed in the relationship between the concentration of methadone and its pharmacological effect when measuring outcomes such as pain relief,[7,13,102-105] quality-of-life scores, mood states, or withdrawal symptoms.[106-108] Several factors have been identified as potential causes for this large interindividual variability, with genetic polymorphism in the gene coding for the μ-opioid receptor (*OPRM1*) as a primary contributor. About 100 variants have been identified in the *OPRM1* gene,[109] with more than 20 variants producing amino acid changes and having polymorphic frequencies of >1%.[110] The most commonly identified SNP is 118A>G (allele frequency 1–48%, ethnicity dependent) causing an amino acid exchange at position 40 from asparagine to aspartate (Asn40Asp), leading to the loss of putative *N*-glycosylation sites in the extracellular receptor region. The affinity of β-endorphin was 3.5-fold higher for this mutated receptor than for wild-type receptors in transfected AV-12 cells,[111] whereas the affinity of other endogenous opioids (met- and leu-enkephalin, endomorphin-1, -2) and the exogenous opioids (morphine, fentanyl, methadone, and naloxone) showed no difference.[67,112]

Moreover, no differences in receptor signalling between mutated and wild-type receptors have been observed when measured by (D-Ala^2,N-Mephe^4,Gly-ol^5)-enkephalin (DAMGO)-stimulated GTPγS binding or cAMP accumulation in different cell lines.[112,113] Zhang et al.,[114] using human brain tissue and transfected Chinese hamster ovary (CHO) cells, reported that the *OPRM1* 118G allele caused lower mRNA and OPRM1 protein levels.

In addition, there have been several studies examining the functional consequences of *OPRM1* 118A>G in healthy subjects, and in patients receiving various opioid drugs including morphine, morphine-6-glucuronide, or alfentanil.[115,116] Although inconsistent findings have been reported, there is more evidence indicating that 118A>G causes a decreased opioid effect (miosis, response to experimental pain, respiratory depression) and increased opioid dosage requirements in patients.[117-119] Moreover, it has been found that 118A>G protects against opioid adverse effects.[120] In a recent study of 51 healthy subjects, using pupil size (miosis) as the response parameter, Lotsch et al.[70] investigated the effect of (*R*)-methadone and demonstrated that carriers of the variant 118G allele had a 1.74-fold lower miotic potency compared with non-carriers. The results indicate that the decreased opioid potency at mutated receptor (Asp40 variant) does also apply to methadone.

In addition to the 118A>G SNP, *OPRM1* 802T>C, which encodes Ser268Pro mutant μ-opioid receptors (intracellular receptor portion), results in altered receptor desensitization and receptor signalling, with decreased G-protein coupling.[121] The affinity for morphine, deprenorphine, DAMGO, and β-endorphin was not changed, but the potency and efficacy of DAMGO, β-endorphin, and morphine were greatly reduced.[113] Other mutations affecting the intracellular receptor portion, such as the 779G>A SNP (encoding Arg260His receptors) and the 794G>A SNP (encoding Arg265His receptors) have been associated with reduced spontaneous receptor signaling, but the association between 779G>A, 794G>A, and opioid potency has not yet been shown.[122]

Therefore, evidence points to the 118A>G polymorphism as being potentially important for opioid therapy. Other mutations in the *OPRM1* gene alter receptor function; however, these are either rare or the current knowledge about their molecular consequences is insufficient to draw conclusions about their probable therapeutic importance.

5. Contribution of Other Receptors

The anti-nociceptive effect of methadone is mediated not only by agonism at the μ-opioid receptor but also by antagonism at the NMDA receptor, which plays an important role in pain transmission.[123-125] Methadone is a noncompetitive NMDA antagonist. It is interesting that NMDA antagonism attenuates and reverses the development of tolerance to morphine without altering its analgesic properties.[123] Genetic polymorphisms in the genes coding for NMDA ion channels have been described, but no relationship to methadone effect has been reported.[126,127]

Methadone is also a strong inhibitor of serotonin and norepinephrine uptake, which might also contribute to its anti-nociceptive activity.[128] Genetic polymorphisms in the gene coding for serotonin receptors, transporters, or for enzymes responsible for neurotransmitter metabolism might be modifiers of methadone action, although the related evidence is lacking.

6. Interactions of Methadone with Other Medications

There is large interindividual variability in the pharmacokinetics and pharmacodynamics of methadone. Besides the above-mentioned genetic polymorphisms in CYP genes, *ABCB1* (encoding the P-gp transporter), and *OPMR1* (encoding the μ-opioid receptor), induction and inhibition of methadone metabolism by coadministered medications are additional factors explaining this variability.

Methadone is metabolized by CYP3A4, CYP2B6, and CYP2D6. As most drugs are substrates, inducers, or inhibitors of these isoenzymes (tables IV and V), drug-drug interactions involving methadone can readily occur. Antituberculosis (rifampin [rifampicin], rifabutine) and anticonvulsant (phenobarbital, phenytoin, carbamazepine) agents are classical CYP3A4 inducers and enhance methadone metabolism, leading to poor analgesia and, possibly, to withdrawal symptoms.[129-134] The antifungal agent fluconazole, an inhibitor of several CYP enzymes including

Table IV. Substrates, inhibitors and inducers of cytochrome P450 (CYP) 3A4, CYP2D6, and CYP2B6

Drug/substance	CYP3A4			CYP2D6			CYP2B6		
	substrate	inhibitor	inducer	substrate	inhibitor	inducer	substrate	inhibitor	inducer
Alprazolam	√								
Alprenolol				√					
Amiodarone	√				√				
Amitriptyline	√								
Amlodipine	√								
Amphetamines				√					
Atenolol				√					
Barbiturates			√						
Buproprion					√		√		
Carbamazepine	√		√						√
Cimetidine		√			√				
Cinnarizine				√					
Ciprofloxacin		√							
Chlorimipramine (Clomiprimine)					√				
Chlorpromazine				√					
Clarithromycin	√	√							
Clozapine	√			√					
Clonazepam	√								
Codeine				√					
Cortisol	√								
Cyclophosphamide							√		
Cyclosporine	√								
Desmethylcitalopram				√					
Dexamethasone	√		√						
Dextromethorphan				√					
Diltiazem	√	√							
Efavirenz			√				√		√
Encainide				√					
Erythromycin	√	√							
Ethinylestradiol	√								
Ethylmorphine				√					
Felbamate			√						
Flecainide				√					
Fluconazole		√							
Flunarizine									
Fluoxetine		√		√	√				
Fluvoxamine		√		√	√				
Grapefruit juice		√							

Continued next page

Table IV. Contd

Drug/substance	CYP3A4			CYP2D6			CYP2B6		
	substrate	inhibitor	inducer	substrate	inhibitor	inducer	substrate	inhibitor	inducer
Haloperidol	√			√	√				
Hypericum			√						
Ifosfamide							√		
Imipramine	√								
Indinavir		√							
Josamycin		√							
Ketoconazole		√							
Levopromazine					√				
Lidocaine	√								
Methadone				√	√		√		
Metoprolol				√					
Mianserine				√					
Midazolam	√								
Moclobemide					√				
Nefazodone	√	√		√					
Nelfinavir		√							
Nevirapine			√						√
Nifedipine	√								
Nimodipine	√								
Norfloxacin		√							
Norfluoxetine		√		√	√				
Oxcarbazepine			√						
Paroxetine		√		√	√				
Perphenazine				√					
Phenobarbital									√
Phenytoin			√						√
Phosphophenytoin			√						
Propranolol				√					
Propafenone				√					
Quinidine	√				√				
Rifampin			√						√
Risperidone	√		√	√					
Ritonavir		√	√		√				√
Sertraline	√			√	√				
Sparteine				√					
Terfenadine	√								
Testosterone	√								
Theophylline	√								
Thiotepa								√	

Continued next page

Table IV. Contd

Drug/substance	CYP3A4			CYP2D6			CYP2B6		
	substrate	inhibitor	inducer	substrate	inhibitor	inducer	substrate	inhibitor	inducer
Thioridazine				√	√				
Ticlopidine								√	
Timolol				√					
Topiramate	√		√						
Tramadol	√			√					
Trazodone				√					
Triazolam	√								
Troleandomycin	√								
Venlafaxine	√	√							

CYP3A4, increases serum methadone AUC and mean peak and trough concentrations.[135] Other CYP3A4 inhibitors, such as itraconazole,[136] may also decrease methadone clearance and, thus, increase serum concentrations of methadone.

MMT is the treatment of choice for heroin addicts who are HIV-positive; therefore, the most frequent and clinically most important interactions are those between methadone and antiretroviral drugs. The main drug interactions with methadone are reported in table VI.

In some cases, the consequences of drug interactions do not occur at initiation of a CYP active drug but rather at the discontinuation of such an agent. For example, when a potent inducer such as rifampin or carbamazepine is discontinued, the patient will become a relatively slow metabolizer as the CYP3A4 pathway falls back to its normal metabolic rate, and what was previously an adequate dose of methadone may now become excessive, leading to clinically significant sedation and respiratory depression.

Drug interactions may occur independently of the CYP system. As methadone is transported by P-gp, induction or inhibition of P-gp significantly modifies the pharmacological properties of methadone. For example, coadministration of P-gp inducers such as rifampin and hypericum (St John's wort) caused a decrease in plasma methadone concentration, associated with a substantial increase in withdrawal symptoms.[129,137] Valspodar increased the bioavailability of methadone as a consequence of P-gp inhibition, which resulted in an increased analgesic effect of methadone.[138] P-gp has a wide range of substrates, and coadministration of other P-gp substrates may also lead to methadone interactions. Table V lists the inducers, inhibitors, and substrates of P-gp.

In addition, many medications interact with methadone via their effect on μ-opioid receptors and should be eliminated from a patient's regimen at the risk of inducing withdrawal syndromes. Partial agonist analgesics (buprenorphine) and mixed agonist-antagonist analgesics (butorphanol, dezocine, nalbuphine, nalorphine, pentazocine) should not be used in patients undergoing MMT because they can displace methadone from μ-opioid receptors.

7. Clinical Use of Methadone

Methadone has been used since the 1960s for the stabilization and maintenance of patients with addictive disorders.[152] Over the past 10 years, interest in the use of methadone for pain treatment has increased. Methadone has been established as an inexpensive and effective agent in treating cancer pain. In recent years, the use of methadone in the treatment of neuropathic pain has been highlighted because of its additional mechanism of action as an NMDA-receptor antagonist.[153] The relationships between dose, plasma levels and effects are not clearly defined, and an optimum range of therapeutic concentrations has not yet been identified. Several studies demonstrate that methadone doses ranging from 60 to 100 mg/day are effective in the majority of patients; however, it is now increasingly acknowledged that doses in excess of 100 mg/day may be required for the optimal treatment of some patients.[12] In practice, although 100 mg/day is considered a maximum by many physicians, doses of >100 mg/day are currently being used in an increasing number of centers. Studies suggest that a blood concentration of 400 ng/mL is considered the optimal therapeutic concentration, and that results under 100 ng/mL are considered clinically ineffective; methadone concentrations above 1000 ng/mL are frequently associated with drug toxicity.[154]

Interestingly, because of the high interindividual variability of methadone blood concentrations for a given dose, achieving concentrations of methadone ~250 ng/mL theoretically requires doses of racemic methadone as low as 55 mg/day or as high as 921 mg/day in a 70-kg patient without any comedication.[155] Therefore, individualizing dosage regimens of methadone is necessary. Therapeutic drug monitoring (TDM) of methadone is not needed in every patient, as a dose titration based on clinical response (i.e. on symptoms of overdose or withdrawal syndrome) is sufficient.[156] However, TDM of methadone could be useful and it is recommended in selected situations, for example, when doses in excess of

Table V. Substrates, inhibitors, and inducers of p-glycoprotein with clinical relevance

Drug/substance	Substrate	Inhibitor	Inducer
Amiodarone		√	
Amitriptyline	√		
Atorvastatin		√	
Bepridil		√	
Chlorpromazine	√	√	
Cyclosporine			√
Dexamethasone	√		√
Digoxin	√		
Diltiazem		√	
Dipyridamole		√	
Domperidone	√		
Erythromycin		√	
Fentanyl	√		
Felodipine		√	
Fexofenadine	√		
Fluphenazine		√	
Hypericum (St John's Wort)			√
Itraconazole	√		
Indinavir	√		
Ketoconazole		√	
Loperamide	√		
Methadone	√		
Morphine	√		√
Nifedipine			√
Nortriptyline	√		
Olanzapine	√		
Ondansetron	√		
Phenobarbital			√
Phenothiazine			√
Phenytoin	√		
Propranolol		√	
Quetiapine	√		
Quinidine		√	
Rifampin			√
Risperidone	√		
Ritonavir		√	
Tamoxifen		√	
Valspodar		√	
Verapamil		√	
Vitamin E		√	
Yohimbine			√

100 mg/day are given to a patient, when treatment failure (persistence of withdrawal symptoms or intake of illicit opioids) is observed, or when comedication is introduced. Trough plasma concentrations of 400 ng/mL for (*R,S*)-methadone, or preferably of 250 ng/mL for (*R*)-methadone might be used as target values for TDM.[155] Patients on higher doses of methadone may develop a prolonged QT interval, which may then lead to the development of torsade de pointes and sudden death.[157]

Recent studies report that the number of methadone-induced deaths is increasing; in the US, the number of deaths attributed to methadone rose from 790 in 1999 to 2990 in 2003.[158] The risk appears to be greatest under the following conditions: IV administration of methadone, oral administration of doses >200 mg/day, and medical conditions or medications predisposing patients to QTc interval prolongation.[28,159] Therefore, electrocardiogram (ECG) monitoring and vigilance are recommended when dealing with patients receiving high doses or IV administration of methadone. However, the administration of methadone is not considered to require a preliminary ECG check, and it does not seem justified to recommend it on a general basis unless the drug is given to patients with known or suspected QT prolongation or patients with multiple risk factors for QTc prolongation.

The possibility that clinically important interactions occur when methadone is taken concomitantly with other drugs is substantial. They can have important consequences such as precipitation of withdrawal symptoms or relapse. Physicians must therefore carefully follow these patients in order to avoid, or at least to notice and treat in time, such interaction. In addition, caution is advised when switching patients to methadone, especially from high doses of previous opioids, because of its variable conversion ratio and the potential for delayed toxicity associated with its long half-life (see tables II and III).[160]

8. Conclusion

Genetic polymorphism and comedication are recognized as important determinants of interindividual variability in methadone pharmacokinetics. The clinician must be aware of the pharmacokinetic properties and pharmacological interactions of methadone in order to personalize methadone administration. Genetic polymorphism is the cause of high interindividual variability of methadone blood concentrations for a given dose: in order to obtain blood concentrations of methadone 250 ng/mL, doses of racemic methadone as low as 55 mg/day or as high as 921 mg/day in a 70-kg patient without any comedication may be required.

Genetics are not the only cause of interindividual variability, and it is important not to forget the other common causes, such as comedication, underlying diseases, environmental and biologic factors that will contribute to variability in methadone response. In practice, pharmacogenomics may explain, to some extent, drug response and toxicity in patients but their utility in daily practice is

Table VI. Main drug interactions with methadone

Agent	Effect on methadone	Possible mechanism	References
Psychotropic and anticonvulsant drugs			
Fluoxetine	Increased plasma concentration	Inhibition of CYP2D6	65,139
Paroxetine	Increased plasma concentration	Inhibition of CYP2D6	33
Fluvoxamine	Increased plasma concentration	Inhibition of CYP2C19, CYP3A4, and CYP2C9	65,139
Carbamazepine	Decreased plasma concentration	Induction of CYP3A4	132
Phenytoine	Decreased plasma concentration	Induction of CYP3A4	133
Phenobarbital	Decreased plasma concentration	Induction of CYP3A4	140
Antifungal and antibacterial drugs			
Ketoconazole	Increased plasma concentration	Inhibition of CYP3A4	141
Fluconazole	Increased plasma concentration	Inhibition of CYP3A4	142
Erythromycin	Increased plasma concentration	Inhibition of CYP3A4	143
Rifampicin	Decreased plasma concentration	Induction of CYP3A4	129
Antiretroviral drugs			
Abacavir	Decreased plasma concentration	Unclear	144
Amprenavir	Decreased plasma concentration	Induction of CYP3A4	144
Efavirenz	Decreased plasma concentration	Induction of CYP3A4	145,146
Indinavir	Increased plasma concentration	Unclear	147
Nelfinavir	Decreased plasma concentration	Induction of CYP3A4	148
Ritonavir	Decreased plasma concentration	Induction of CYP3A4	148,149
Nevirapine	Decreased plasma concentration	Induction of CYP3A4	150,151

not necessary. In the future, pharmacogenomics, at a limited level, can also be expected to facilitate individualized methadone therapy.

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