

Pharmacokinetic and Pharmacogenetic Factors Influencing Methadone Plasma Levels

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Summary

Methadone is widely used as a maintenance treatment for opiate addiction. Methadone plasma levels vary widely for a given dose, so contributing to interindividual variability in response to methadone maintenance treatment. Until recently, the relative in vivo involvement of various cytochrome P450 (CYP) isoforms in methadone pharmacokinetics had been unclear. A recent large-scale pharmacogenetic study with patients in methadone maintenance treatment has now demonstrated that CYP3A4 and CYP2B6 are the major cytochrome P450 isoforms with a major involvement in methadone metabolism, while CYP2D6 only contributes to a minor extent. In addition, P-glycoprotein, a transmembrane efflux protein, is also involved in methadone kinetics.

Key Words: Methadone Treatment - Pharmacokinetics -
Cytochrome P450 Enzymes - P-glycoprotein

Introduction

Methadone, which is widely used as a maintenance treatment for opiate addiction, is a synthetic μ -opioid receptor agonist. It is marketed in almost all countries as a racemic mixture of (R)- and (S)-methadone, although most of the opioid effect is produced by the (R)-enantiomer⁽²⁶⁾. The metabolism of methadone is mediated by cytochrome P450 (CYP) enzymes, mostly leading to the inactive 2-ethylidene-1,5-dimethyl-3,3-diphe-

nylpyrrolidine (EDDP) by the N-demethylation pathway⁽²⁸⁾. Very large interindividual variations in methadone plasma levels after a given dose had already been demonstrated; these variations partly account for the high levels of interindividual variability found in response to treatment⁽⁸⁾. Wide-ranging variations in the relationship between dose and plasma concentration are typical of drugs that are metabolized and/or transported by polymorphic proteins⁽⁸⁾. In addition, many patients in methadone maintenance treatment (MMT) take concomitant medication(s); this may well influence methadone kinetics, so accentuating interindividual variability.

Cytochrome P450 Family

The interindividual variability of the activity of CYP enzymes, along with their potential for inductions and inhibitions, is probably responsible for a large proportion of the variations in methadone kinetics and observed plasma levels⁽⁸⁾. Several in vitro and in vivo studies have demonstrated the involvement of different CYPs in methadone metabolism. For a long time, CYP3A4 was thought to be the main CYP isoform involved in methadone metabolism, together with CYP2D6 and possibly CYP1A2, CYP2C8, CYP2C9 and CYP2C19^(1,7,8,11,13,15,17,21,32), but in vivo the involvement of each of these was unclear. More recently, two in vitro studies demonstrated that CYP3A4 and CYP2B6 were the main enzymes involved in the methadone metabolism^(13,17). Interestingly, in one of these studies, the enantiomers of methadone were evaluated separately and a stereoselectivity was observed for CYP2B6 in favour of the (S)-enantiomer, and for CYP2C19 towards the (R)-enantiomer, whereas CYP3A4 display no stereoselectivity⁽¹³⁾. In a large-scale pharmacogenetic study, we were able to confirm in vivo the impact of CYP2B6 on (S)-methadone plasma levels⁽⁴⁾. The MMT patients carrying a *CYP2B6* *6/*6 genotype presented significantly higher trough (S)-methadone plasma levels than the non-carriers of allele *6 (209 and 105 ng*kg/ml*mg, respectively; p=0.0004), whereas the impact on (R)-methadone plasma levels was not significant⁽⁴⁾. As the (S)-enantiomer makes no contribution to the μ -opioid receptor activation, the *CYP2B6**6 allele does not influence responses to treatment⁽⁴⁾.

As to CYP3A4, its involvement in the methadone metabolism was most strongly suggested by interaction studies with CYP3A4 inducers and/or inhibitors^(2, 8, 17). It should be noted that one study showed a higher level of CYP3A4 activity measured by the midazolam phenotyping test in 32 MMT patients receiving high methadone doses; this high level of activity may contribute to the need for high doses⁽²⁷⁾. On the other hand, based on the lack of specificity of the inducers and/or inhibitors used in these interaction studies, it was concluded that CYP3A4 may play only a minor role in the methadone metabolism in vivo⁽¹⁷⁾, leading to a predominant role to CYP2B6 and a possible role for intestinal transporters⁽¹⁶⁾.

The expression and activity of CYP3A4 vary significantly between individuals⁽³⁴⁾; most of this variability is thought to be genetically determined. However, the low allelic frequency of the functional polymorphisms of CYP3A4 cannot account for the

variations that have been observed^(10,34). Therefore, a phenotyping test better reflects its activity than the genotyping of the described alleles. For example, midazolam is a substrate of both CYP3A4 and CYP3A5, and its oral administration makes it possible to measure both intestinal and hepatic CYP3A activity⁽⁹⁾. Midazolam's metabolic ratio was shown to correlate well with midazolam clearance; this allows it to be used as a marker of CYP3A activity⁽⁹⁾. We recently demonstrated that midazolam's metabolic ratio correlated with (R)-, (S)-, and (R,S)-methadone plasma levels, so confirming the *in vivo* involvement of CYP3A in the methadone metabolism⁽⁵⁾. In addition, we found that CYP3A activity failed to display stereoselectivity in favour of any of the enantiomers of methadone⁽⁵⁾, as might have been expected from an enzyme with such a wide range of substrates; this finding confirmed previous *in vitro* results^(11,13).

Unlike CYP3A4, the hepatic expression of CYP3A5 is distributed bimodally, indicating the existence of a polymorphism⁽³⁴⁾. *CYP3A5*3* causes alternative splicing and protein truncation, resulting in an absence of CYP3A5 in most Caucasians⁽¹⁸⁾. *In vitro*, CYP3A5 was not shown to be involved in the methadone metabolism^(15,17), but it can constitute as much as 50% of the total hepatic CYP3A content in people who express it⁽¹⁸⁾, which makes it likely that it contributes to the interindividual variability of the methadone metabolism. We recently discovered that methadone plasma levels did not differ according to the presence or the absence of CYP3A5, so confirming that CYP3A5 is not involved in the methadone metabolism⁽⁵⁾.

As regards CYP2D6, only a minor involvement in the methadone metabolism was demonstrated *in vitro*^(13,15,17,21,32), but observed interactions between methadone and CYP2D6 inhibitors provided an indication of a more important involvement^(1,6), maybe by another pathway than N-demethylation⁽⁸⁾. A previous *in vivo* study found a significant difference in methadone concentrations corrected by dose and weight between poor metabolizers (PMs), extensive metabolizers (EMs) and ultrarapid metabolizers (UMs)⁽⁷⁾ of CYP2D6. This influence of the UM genotype on trough methadone plasma levels has recently been confirmed⁽⁵⁾. On the other hand, the PM status of CYP2D6 had no influence on methadone plasma levels, possibly due to a compensatory activity by other CYP isoforms in CYP2D6 PMs⁽⁵⁾.

As to CYP1A2, *in vitro* it was not shown to be involved in the methadone metabolism^(13,15,15) or was shown to have only a minor role^(21,32). But a previous report on MMT patients who were smokers revealed that the heavy smokers were those most likely to report problems arising from not feeling 'held' by their methadone dose⁽²⁹⁾. As CYP1A2 is induced by cigarette smoking, these differences may have been caused by the CYP1A2-mediated metabolism, most likely by another metabolic pathway than N-demethylation to EDDP⁽⁸⁾. The *CYP1A2*1F* allele was proposed as possibly presenting a higher inducibility, on the grounds that a significant difference in CYP1A2 metabolic activity between the genotypes was only observed in smokers⁽²⁴⁾. However, we found no influence of the *CYP1A2*1F* genotype on methadone plasma levels, which suggests that this isozyme does not contribute to the methadone metabolism⁽⁵⁾.

Lastly, certain *in vitro* studies have shown the involvement of CYP2C9 and CYP2C19

in the methadone metabolism^(11, 13, 17, 21). In particular, one of these studies showed an important involvement of CYP2C19, with a stereoselectivity favouring the active (R)-enantiomer for this isoform⁽¹³⁾. But in vivo, for both isozymes, the PM status was not found to influence methadone plasma levels⁽⁴⁾.

P-Glycoprotein

Several in vitro and animal models have been used to demonstrate that methadone is a substrate of P-glycoprotein (PGP)^(3, 22, 23, 30, 33), a transmembrane efflux transporter belonging to the ATP-binding cassette (ABC) family, and encoded by the *ABCB1* gene. The expression of PGP in various human tissues, including the intestine, liver, kidneys, testes and blood-brain barrier⁽²⁰⁾, demonstrates its protective role against the potentially toxic accumulation of xenobiotics, in enhancing of their elimination and limiting of their distribution in the body⁽¹²⁾. One important function of PGP is its limitation of the access of xenobiotics to the brain, which has been demonstrated in vivo by studies on PGP-deficient mice⁽²⁵⁾. The presence of PGP in the blood-brain barrier, intestine and kidneys is therefore of special interest during methadone treatment. In particular, methadone distribution to the brain has been shown to be regulated by PGP in mice and rats^(23, 33). Furthermore, the intestinal absorption or renal elimination of methadone may be related to PGP intestinal or renal content. This probable role of PGP in the intestinal disposition of methadone was suggested in a study on healthy subjects⁽¹⁶⁾.

Several single nucleotide polymorphisms (SNPs) of the *ABCB1* gene have been reported, in particular the synonymous *3435C>T* SNP, which has been associated with lower PGP expression⁽¹⁴⁾. Interestingly, it has recently been demonstrated that the *3435TT* genotype is associated with a fall in PGP expression due to a decrease in mRNA stability⁽³¹⁾. In one study in which 51 healthy volunteers took a single methadone dose, no influence of *ABCB1 2677G>T* and *3435C>T* was observed on methadone AUC and peak plasma levels⁽¹⁹⁾. But in studying steady-state MMT patients, we found that the *ABCB1 3435C>T* SNP had an influence on trough but not on peak methadone plasma levels⁽⁵⁾. Stereoselectivity in PGP transport was not expected, as PGP can transport a wide range of different chemical substances. Despite this, a study that quantified the enantiomers of methadone to assess stereoselectivity in methadone transport using *Abcb1a* knockout mice observed an apparently lower brain access for (S)-methadone than for (R)-methadone, so suggesting stereoselectivity in the activity of mouse PGP⁽³³⁾. In studying MMT patients, however, we did not find differences in the influence of the *ABCB1 3435C>T* genotypes on (R)- and (S)-enantiomer plasma levels.

In summary, in vivo and in vitro results converge in identifying CYP3A4 and CYP2B6 as the major CYP isoforms involved in the methadone metabolism, and in indicating that CYP2D6 only contributes to a minor extent. As several CYP proteins contribute simultaneously to methadone kinetics, a decreased function of one of them does not lead to a major effect on methadone plasma levels. The genetic polymor-

phisms of *ABCB1* also contribute to a small extent to the interindividual variability of methadone kinetics.

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