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Is toxicity of PMMA (paramethoxymethamphetamine) associated with cytochrome P450 pharmacogenetics?

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ABSTRACT

In 2010–2013, 29 fatal intoxications related to the designer drug paramethoxymethamphetamine (PMMA, 4-methoxymethamphetamine) occurred in Norway. The current knowledge about metabolism and toxicity of PMMA in humans is limited. Metabolism by the polymorphic cytochrome P450 (CYP) 2D6 enzyme to the psychoactive metabolite 4-hydroxymethamphetamine (OH-MA), and possibly by additional enzymes, is suggested to be involved in its toxicity. The aim of this work was to study the association between CYP genetics, PMMA metabolism and risk of fatal PMMA toxicity in humans. The frequency distribution of clinically relevant gene variants of *CYP2D6*, *CYP2C9*, *CYP2C19* and *CYP3A5*, and the phenotypic blood CYP2D6 metabolic ratio (OH-MA/PMMA) in particular, were compared in fatal PMMA intoxications (n = 17) and nonfatal PMMA abuse controls (n = 30), using non-abusers (n = 305) as references for the expected genotype frequencies in the Norwegian population. Our study demonstrated that the CYP2D6 enzyme and genotype are important in the metabolism of PMMA to OH-MA in humans, but that other enzymes are also involved in this biotransformation. In the fatal PMMA intoxications, the blood concentrations of PMMA were higher and the CYP2D6 metabolic ratios were lower, than in the nonfatal PMMA abuse controls (median (range) 2.1 (0.03–5.0) vs 0.3 (0.1–0.9) mg/L, and ratio 0.6 (0.0–4.6) vs 2.1 (0.2–7.4) p = 0.021, respectively). Overall, our findings indicated that, in most cases, PMMA death occurred rapidly and at an early stage of PMMA metabolism, following the ingestion of large and toxic PMMA doses. We could not identify any genetic *CYP2D6*, *CYP2C9*, *CYP2C19* or *CYP3A5* predictive marker on fatal toxicity of PMMA in humans. The overrepresentation of the CYP2D6 poor metabolizer (PM) genotype found in the nonfatal PMMA abuse controls warrants further investigations.

1. Introduction

In 2010–2013, a cluster of 29 fatal poisonings related to the toxic designer drug paramethoxymethamphetamine (PMMA, 4-methoxymethamphetamine) was revealed in Norway [1]. PMMA is a ring-substituted monomethoxy methamphetamine (MA) derivative, and an N-methylated derivative of the pharmacologically similar designer drug paramethoxyamphetamine (PMA, 4-methoxyamphetamine) (Fig. 1). The ring-substitution adds potent serotonergic, hallucinogenic and MDMA-like (ecstasy, 3,4-methylenedioxymethamphetamine) properties to methamphetamine/amphetamine drugs [2]. PMMA and PMA are substitute drugs occasionally found in powder or tablets illegally sold as "amphetamine" or "ecstasy". These drugs act by increasing the release and inhibiting the reuptake of serotonin and by reversibly inhibiting the enzyme monoamine oxidase A (MAO-A) [3,4]. The toxicity of PMMA is regarded as substantially higher than for amphetamine, methamphetamine and MDMA [5], as indicated by 131 fatal and 31 nonfatal poisonings associated with the abuse of PMMA worldwide [1,6–10]. The toxicity of PMMA is positively correlated with the PMMA dose and the blood drug level, but the existing literature indicates that certain human subjects may have an increased risk of PMMA toxicity [1]. This has also been suggested for other designer amphetamines like 4-methylthioamphetamine (4-MTA) [11].

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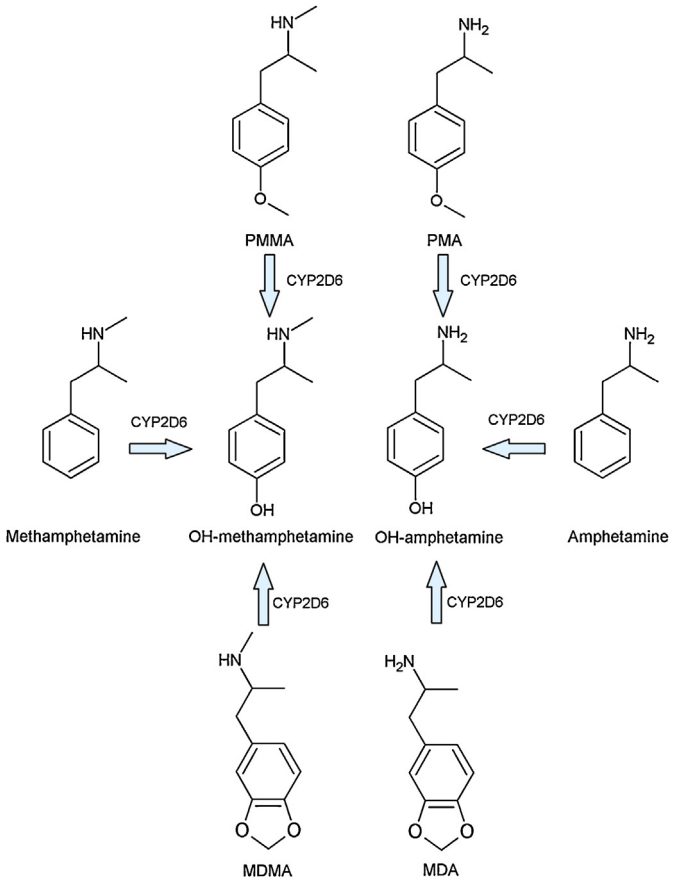

Fig. 1. Chemical structure and major metabolic pathways of PMMA, PMA, methamphetamine, amphetamine and MDMA; PMMA, paramethoxymethamphetamine, PMA, paramethoxyamphetamine, MDMA, 3,4-methylenedioxymethamphetamine, CYP2D6, cytochrome P450 2D6.

Genetic and phenotypic variability in the PMMA metabolism might be relevant vulnerability traits.

The current knowledge about the PMMA metabolism and toxicity in humans is limited. The available information about metabolism is based solely on two rat studies [12,13], one study using human liver microsomes [14] and one urine sample from a single individual [12]. As is the case with many synthetic amphetamine analogues, PMMA metabolism probably occurs mainly through O-demethylation via the polymorphic cytochrome P450 (CYP) 2D6 enzyme to the psychoactive and potentially toxic metabolite 4-hydroxymethamphetamine (OH-MA, pholedrine) followed by conjugation with glucuronide or sulphate [14,15]. N-demethylation to PMA is a minor pathway, mainly by other CYP enzymes and MAO-A [16]. PMA is the only metabolite unique for PMMA, as the other PMMA metabolites are also formed from MDMA [17] and methamphetamine [18] by the same enzymes (Fig. 1).

Due to genetic variation and pharmacological interactions, CYP2D6 catalytic activity varies considerably in all populations examined. Based on *CYP2D6* genotype, subjects can be classified into four CYP2D6 phenotype categories: poor (PM), intermediate (IM), extensive (EM) and ultrarapid metabolizers (UM) [19]. It has been suggested that CYP2C isoforms might be involved in the deamination of amphetamines and that *CYP2C* gene variants might contribute to interindividual differences in drug action [16]. Also CYP isoenzymes of the 1A, 2B and 3A subfamilies have the capacity to metabolize ring-substituted amphetamines, possibly of particular importance in CYP2D6 poor metabolizers [20].

Previous reports on the association between CYP2D6 genotypes, drug metabolism and the risk of stimulant drug toxicity have been conflicting. Early studies on PMA, MDMA and other ring-substituted amphetamines suggested that the CYP2D6 PM phenotype could be a predisposing factor for acute drug toxicity, due to the high toxicity of these parent drugs in high doses [21–23]. However, later studies found no evidence for this [24–27]. Instead, it was suggested that the CYP2D6 UM phenotype might predispose users to toxic effects, due to toxic metabolites [11,16,28–32]. Active metabolites were proposed to be involved in the toxicological effects of some amphetamines, particularly hydroxy- and dihydroxy- (catechol) metabolites or their reactive conversion products [33–35]. It has been questioned whether systemic metabolism is important for PMMA toxicity, as has been reported for MDMA where this is proposed to be a crucial step for toxicity [32].

The aim of the present work was to study the association between CYP genetics, PMMA metabolism and the risk of fatal PMMA toxicity in humans, by comparing the frequency distribution of clinically relevant gene variants of *CYP2D6*, *CYP2C9*, *CYP2C19* and *CYP3A5* enzymes, and the phenotypic blood CYP2D6 metabolic ratio (OH-MA/PMMA) in particular, in fatal PMMA intoxications and nonfatal PMMA abuse controls, using non-abusers as references for the expected genotype frequencies in the Norwegian population.

2. Material and methods

Our cases included all of the fatal PMMA intoxications recorded in Norway during the study period from June 2010 to February 2013. Our control group included nonfatal PMMA-related drug abuse cases recorded in the Forensic Toxicology Database at the Norwegian Institute of Public Health (NIPH) during the same time period. As references for CYP genotype frequencies in the Norwegian population with no known drug abuse, we included healthy living blood donors and natural death cases not related to drug use, reference groups C2 and C3, respectively (Table 1). The exclusion and inclusion criteria for these four groups are described below. The study was approved by the Regional Committee for Medical and Health Research Ethics, and by the Higher Prosecution Authority as the owner of the forensic samples.

2.1. Inclusion in the study

2.1.1. Fatal PMMA intoxications

During the study period of almost three years, PMMA was detected in post-mortem blood samples from 27 forensic autopsy cases at NIPH and in 2 cases from Mid-Norway (personal communication with Lars Slørdal, St. Olavs Hospital, Trondheim). Based on our study's inclusion and exclusion criteria, the forensic toxicology results and the forensic pathologist's classification of cause of death, 12 of these 29 cases were not found to be eligible for inclusion in our study, due to violent cause of death ($n = 2$), no remaining blood ($n = 2$), genotyping failed ($n = 2$) or heroin/morphine being judged as the major cause of death ($n = 6$). These 17 fatal PMMA intoxications constituted our cases. The influence of other drugs as a cause of death is illustrated in Table 2. Based on the available information including the pathologist's conclusion, the fatal PMMA intoxications were categorized as "Fatal PMMA only" with no major contribution of other drugs, and as "Fatal PMMA polydrug" intoxications when a significant concentration of other psychostimulant or sedative drugs was detected in blood. According to the existing literature, we defined a blood amphetamine or methamphetamine concentration above 0.2 mg/L (i.e. lower recreational level) to be "significant" [36] (analytical cut-off 0.03 mg/L), or any concentration of the more toxic substances MDMA, cocaine or ethanol above the analytical cut-off applied in