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3,4-Methylenedioxymethamphetamine (MDMA) interacts with therapeutic drugs on CYP3A by inhibition of pregnane X receptor (PXR) activation and catalytic enzyme inhibition

Irene Antolino-Lobo[a,b,*], Jan Meulenbelt[a,b,c], Sandra M. Nijmeijer[a], Roel F. Maas-Bakker[d], Irma Meijerman[d], Martin van den Berg[a], Majorie B.M. van Duursen[a]

[a] Institute for Risk Assessment Sciences, Utrecht University, Utrecht, Netherlands
[b] National Poisons Information Centre, National Institute for Public Health and the Environment, Bilthoven, Netherlands
[c] Division Intensive Care Center, University Medical Center Utrecht, Utrecht, Netherlands
[d] Division of Biomedical Analysis, Department of Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, Netherlands

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ABSTRACT

Metabolism of MDMA (3,4-methylenedioxymethamphetamine, Ecstasy) by the major hepatic drug-metabolizing enzyme cytochrome P450 3A (CYP3A), plays an important role in MDMA-induced liver toxicity. In the present study, we investigated interactions between MDMA and several therapeutic and recreational drugs on CYP3A and its regulator pregnane X receptor (PXR), using a human PXR-mediated CYP3A4-reporter gene assay, rat primary hepatocytes and microsomes. MDMA significantly inhibited hPXR-mediated CYP3A4-reporter gene expression induced by the human PXR activator rifampicin (IC_{50} 1.26 ± 0.36 mM) or the therapeutic drugs paroxetine, fluoxetine, clozapine, diazepam and risperidone. All these drugs concentration-dependently inhibited CYP3A activity in rat liver microsomes, but in combination with MDMA this inhibition became more efficient for clozapine and risperidone. In rat primary hepatocytes that were pretreated with or without the rodent PXR activator pregnenolone 16alpha-carbonitrile (PCN), MDMA inhibited CYP3A catalytic activity with IC_{50} values of 0.06 ± 0.12 and 0.09 ± 0.13 mM MDMA, respectively. This decrease appeared to be due to decreased activation of PXR and subsequent decreased CYP3A gene expression, and catalytic inhibition of CYP3A activity. These data suggest that in situations of repeated MDMA use in combination with other (therapeutic) drugs, adverse drug–drug interactions through interactions with PXR and/or CYP3A cannot be excluded.

1. Introduction

MDMA (3,4-methylenedioxymethamphetamine, "ecstasy") is an illicit drug of abuse with stimulant and hallucinogenic properties. The use of MDMA is mainly associated with nightclubs, dance music and some sub-cultures, and during the past years, a significantly higher level of consumption is being reported among young people (EMCDDA, 2008). Hepatotoxicity has been described as one of the clinical effects of MDMA intoxication (Andreu et al., 1998; Khakoo et al., 1995). Metabolism of MDMA appears to play an important role in MDMA-induced hepatotoxicity and its metabolic mechanism has been the subject of several studies (Antolino-Lobo et al., 2010; Jones et al., 2005; Milhazes et al., 2006). Although most studies focused on cytochrome P450 2D6 (CYP2D6), we and others have shown that cytochrome P450 3A (CYP3A) also plays an important role in MDMA bioactivation and possible MDMA-induced hepatotoxicity (Antolino-Lobo et al., 2010; Kreth et al., 2000).

Members of the CYP3A family play a major role in the biotransformation of various compounds, such as endogenous hormones and xenobiotics, and account for about 60% for the metabolism of clinically used therapeutic drugs. CYP3A enzymes have a large hydrophobic active site and exhibit atypical pharmacokinetics, which strongly suggests the presence of multiple substrate binding sites (Zhou et al., 2005). Further, it displays interindividual variability in its specific activity in the liver and small intestine, the primary

Abbreviations: CYP450, cytochrome P450; PXR, pregnane X receptor; MDMA, 3,4-methylenedioxymethamphetamine; DZ, diazepam; DPH, phenytoin; CLZ, clozapine; RSP, risperidone; FLX, fluoxetine; PX, paroxetine; mCPP, chlorophenylpiperazine; AP, amphetamine; R, rifampicin; PCN, pregnenolone 16alpha-carbonitrile; SFN, sulforaphane; LS180, human colon adenocarcinoma-derived cell line; MTT, 3,(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; α-NF, alpha-naphtoflavone; QUI, quinidine; DDIs, drug–drug interactions.

* Corresponding author at: Institute for Risk Assessment Sciences, Yalelaan 2, 3584 CM Utrecht, Netherlands. Tel.: +31 30 2533631; fax: +31 30 2535077.
E-mail addresses: i.antolinolobo@uu.nl, irene.antolinolobo@hotmail.com (I. Antolino-Lobo).

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sites of drug metabolism (Zhou et al., 2007). Pregnane X receptor (PXR) plays a key role in regulating the expression of CYP3A drug-metabolizing enzymes (Goodwin et al., 1999; Lehmann et al., 1998). Many therapeutic drugs can interact with PXR and consequently modulate CYP3A expression in vitro (Cui et al., 2008; Kliewer et al., 2002; Luo et al., 2002) and in vivo (Hewitt et al., 2007; Kim et al., 2010; Luo et al., 2002). PXR has been proposed to be a protective mediator of liver toxicity by reducing pathophysiological concentrations of toxic bile acids (Staudinger et al., 2001). Also PXR may be considered as a biosensor, acting as a low-affinity receptor with a broad substrate specificity. As such, its biological role is to detect xenobiotics and trigger the production of a wide variety of metabolizing enzymes and transporter proteins (Blumberg et al., 1998; Kliewer et al., 2002). PXR is highly expressed in the liver and intestine, and upon ligand binding it forms a heterodimer with the 9-cis retinoic acid receptor (RXR). This PXR/RXR-dimer is transported to the nucleus where it binds to specific sites on the DNA and regulates, among others, members of the CYP3A family (Kliewer et al., 2002). Activation of PXR occurs in a species- and tissue-specific manner and is even further complicated by the many pharmacophores, various coactivator binding sites and interactions with signal transduction pathways that can regulate PXR activation (Ekins et al., 2007; Lichti-Kaiser et al., 2009).

Modulation of PXR and CYP3A expression and activity by xenobiotics has been suggested to play a major role in drug–drug interactions (DDIs) and adverse drug reactions, which is a major public health problem (Kliewer et al., 2002). DDIs often occur at the level of metabolizing enzymes or nuclear receptors that regulate these enzymes. Inactivation or induction of CYP3A4 may cause severe drug toxicity due to metabolic inhibition or bioactivation of coadministered drugs (Dresser et al., 2000). At present, there is a distinct lack of knowledge regarding the use of MDMA and risk for DDIs, especially with regard to interactions with PXR and/or CYP3A (Li, 2009; Yueh et al., 2005). Besides severe acute clinical effects such as tachycardia, hypertension, hyperthermia, intracranial hemorrhage, serotonin syndrome, and even death, cases of hepatotoxicity due to MDMA ingestion have also been reported. Yet it is probable that many more cases are subclinical and remain undetected. Subacute hepatotoxicity can ultimately lead to severe liver damage after repeated exposure, and MDMA is considered to be a cause of drug-induced liver injury. The absence of information on potential DDIs by MDMA is of toxicological and clinical concern because of the high prevalence of MDMA and other recreational drugs consumption among psychiatric patients (Modestin et al., 1997). Many therapeutic drugs used for treating psychiatric disorders or clinical treatment of MDMA intoxications, such as diazepam, are (partly) metabolized by CYP3A (Spina et al., 2003; Spina and de Leon, 2007) or are agonists for PXR, such as the antiepileptic phenytoin (Luo et al., 2002).

In the present study, we investigated the potential interaction of MDMA with PXR and effects on CYP3A expression and activity in a hPXR-CYP3A4 reporter-based assay, rat primary hepatocytes and liver microsomes. In addition, potential interactions on PXR and CYP3A were studied between MDMA and drugs that are often prescribed to MDMA consumers or used to treat MDMA intoxications such as the anti-anxiety agent/muscle relaxant diazepam, antiepileptic (phenytoin), antipshychotics (clozapine, risperidone), antidepressants (fluoxetine, paroxetine) or other recreational drugs found in rave-party pills (*meta*-chlorophenylpiperazine, caffeine and amphetamine).

2. Materials and methods

2.1. Chemicals and reagents

D,L-Methylenedioxymethamphetamine (MDMA), was obtained from Duchefa-Farma (Haarlem, The Netherlands). D,L-Amphetamine (AP), diazepam (DZ), phenytoin (DPH), clozapine (CLZ), risperidone (RSP), paroxetine (PX), fluoxetine (FLX), meta-chlorophenylpiperazine (mCPP), caffeine, rifampicin (R), ketoconazole, sulforaphane (SFN), 3,(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), alpha-naphtoflavone (α-NF), quinidine (QUI), PCN (pregnenolone 16alpha-carbonitrile) and 8-Br-cAMP (8-bromo-cyclic-AMP) were purchased from Sigma (St. Louis, MO, USA). 7-Benzyloxy-4-(trifluoromethyl) coumarin (BFC) was obtained from BD-Gentest (Woburn, MA). Silencer pre-designed siRNA was purchased from Ambion (Austin, TX, USA).

2.2. hPXR-mediated CYP3A4-reporter gene assay

The CYP3A4 luciferase reporter construct (pGL3-CYP3A4-XREM) was kindly provided by Dr. Christopher Liddle (Westmead Millenium Institute, Westmead, Australia), the nuclear receptor expression vector (pCDG-hPXR) was a generous gift from Dr. Ronald M. Evans (Salk Institute for Biological Studies, La Jolla, CA, USA) described by Blumberg et al. (1998), and the *Renilla* luciferase expression control vector (pRL-TK) was purchased from Promega (Madison, WI, USA). Plasmids were purified using Promega's Pureyield Midi-prep (Madison, WI, USA).

The hPXR-mediated CYP3A4-reporter gene assay was performed as described by Harmsen et al. (2008, 2009). The human colon adenocarcinoma-derived cell line LS180 was purchased from ATCC (Manassas, VA, USA) and cultured according to their instructions. Following overnight transfection, the cells were washed with 200 μl PBS and exposured to the various drugs. Ten μM rifampicin (a known human PXR activator and model inducer of CYP3A4) was used as control for hPXR agonism, while ketoconazole and sulforaphane were used as control compounds for hPXR antagonism. Diazepam, phenytoin, clozapine, risperidone, paroxetine, fluoxetine, mCPP, caffeine and amphetamine were added to the wells individually or in combination with MDMA (0.1 and 1 mM). All chemicals were dissolved in DMSO except for MDMA, which was dissolved in sterile water. The final solvent (DMSO) concentrations did not exceed 0.1% (v/v). The plates were incubated for 48-h at 37 °C with 5% CO_2. Cytotoxicity of all incubations was assessed by measuring the capability of the cells to reduce MTT (3,(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to the blue-colored formazan after a 1-h incubation. Quantification of the formazan dye was measured spectrophotometrically at 595 nm (FLUOstar Galaxy, BMG Labtechnologies). For reporter gene analysis, medium was removed and the cells were washed with PBS and lysed with 25 μl passive lysis buffer (Promega, Madison, USA). The cell lysates (10 μl) were transferred to a white half area 96-well plate (Corning, NY, USA) and the reporter activities of the *Firefly* and *Renilla* luciferases were determined using the Dual-Luciferase™ Reporter (DLR) Assay System (Promega, Medison, WI, USA) according to the manufacturer's instructions. Luminescence was recorded on a Mithras LB940 microplate reader (Berthold Technologies, Bad Wildbad, Germany). The fold of induction was calculated by normalization of the *Firefly*-luciferase signal to the *Renilla*-luciferase signal.

2.3. RNA interference of PXR

The LS180 cells were reversely transfected during 48-h with 10 nM siRNA PXR or 10 nM negative control siRNA according to the manufacturer's protocol for Lipofectamine RNAi Max (Invitrogen, Breda, The Netherlands). The siRNA sequence used for targeting the PXR (sense: cguuuguucgcuuccugagtt; antisense: cucaggaagcgaacaaacgtg) and the negative control (no complementary sequence) were purchased from Ambion (Austin, TX, USA). After the 48-h PXR knockdown, LS180 cells were treated with rifampicin and/or MDMA for 48-h. RNA isolation and amplification are described below (see Section 2.6).

2.4. Primary rat hepatocytes and liver microsomes

Rat hepatocytes were isolated from male Wistar rats using a standard collagenase perfusion method as described previously (Treijtel et al., 2004). Animals received humane care in compliance with the institutional guidelines, under approval of the Animal Ethical Committee according to Dutch law on Use of Experimental Animals. Hepatocytes were plated in 24- or 12-wells plates at a density of 5×10^5 and 1×10^6 cells/well for CYP3A activity and gene expression, respectively. Cells were allowed to attach for 4 h and then exposed to 10 μM PCN, a known rodent PXR activator, or control medium (DMSO 0.1% (v/v)). After 24-h, the cells were washed with warm PBS and medium was replaced with medium containing DMSO or PCN and the test compounds for 24-h.

For microsomal preparations, liver samples were weighed and homogenized in 10 ml Tris–HCl buffer (Tris–HCL 50 mm: 1.15% KCl, pH 7.4) per g tissue, using a Potter–Elvehjem Teflon-glass homogenizer. Afterwards, samples were centrifuged at 9184 × *g* for 25 min at 4 °C. Subsequently, supernatants were pipetted into a clean ultra-centrifuge tube and centrifuged for 75 min at 82,656 × *g* at 4 °C to separate the microsomal (pellet) from the cytosolic (supernatant) fractions. Pellets were resuspended in 1 ml sucrose solution (0.25 M) per g of original tissue. Aliquots of the microsomal fractions were stored at −70 °C until use. Protein contents of isolated microsomes were determined according to the method of Lowry using bovine serum albumine (BSA) as protein standard.