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Short communication

Designer drug 2,5-dimethoxy-4-methyl-amphetamine (DOM, STP): Involvement of the cytochrome P450 isoenzymes in formation of its main metabolite and detection of the latter in rat urine as proof of a drug intake using gas chromatography–mass spectrometry

Andreas H. Ewald [a], Michael Puetz [b], Hans H. Maurer [a,*]

[a] *Department of Experimental and Clinical Toxicology, Institute of Experimental and Clinical Pharmacology and Toxicology, Saarland University, Kirrberger Strasse 1, D-66421 Homburg (Saar), Germany*
[b] *Bundeskriminalamt, KT 34-Forensic Toxicology, D-65173 Wiesbaden, Germany*

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Abstract

The designer drug 2,5-dimethoxy-4-methyl-amphetamine (DOM, STP) is known to be extensively metabolized in various species. The current study showed that cytochrome P450 2D6 was the only isoenzyme involved in formation of the main metabolite hydroxy DOM. In addition, the authors' systematic toxicological analysis (STA) procedure using full-scan GC–MS was suitable to prove an intake of a common drug users' dose of DOM by detection of hydroxy DOM in rat urine. Assuming similar metabolism, the described STA procedure should be suitable for proof of an intake of DOM in human urine. However, DOM and/or other metabolites such as deamino-oxo-hydroxy DOM might be the target analyte in urine of CYP2D6 poor metabolizers.

Keywords: 2,5-Dimethoxy-4-methyl-amphetamine; DOM; STP; Designer drug; GC–MS; CYP2D6

1. Introduction

2,5-Dimethoxy-4-methyl-amphetamine (DOM) is a hallucinogenic drug that was first synthesized in 1964 by Shulgin. In mid-1967, it was distributed under the street name STP, which was said to stand for Serenity, Tranquility, and Peace, but more probably derived from its original cover name "Scientifically Treated Petroleum". It belongs to a group of amphetamines having in common two methoxy groups in position 2 and 5 of the ring and one lipophilic substituent in position 4, for example a halogen (2,5-dimethoxy-4-iodo-amphetamine, DOI; 2,5-dimethoxy-4-bromo-amphetamine, DOB) or a methoxy moiety (2,4,5-trimethoxy-amphetamine, TMA-2). The hallucinogenic properties of these amphetamines seem to be mediated by agonistic and/or antagonistic effects on various serotonin (5-HT) receptors, especially on 5-HT_2 receptor subtypes [1–4]. Shortly after its first synthesis, DOM appeared on the illicit drug market in California, USA. Common drug abusers' doses for DOM ranged from 3 to 10 mg [5]. In 1971 DOM was scheduled by the UN in the convention on psychotropic substances. Further evidence about its popularity among drug abusers can be found, with reservations, on internet web sites (http://www.erowid.org, http://www.lycaeum.org; September 2007) where experience reports and descriptions of DOM have been published.

The metabolism of DOM has been studied in various species such as rabbits [6–9] and rats [10,11]. These metabolism studies have shown that DOM is metabolized by hydroxylation of the 4-methyl moiety partly followed by conjugation or oxidation to the corresponding acid, deamination to the corresponding ketones, partly followed by reduction to the corresponding alcohols, *O*-demethylation, and combinations of these steps. In contrast to other 2,5-dimethoxy-amphetamines such as DOI, DOB, or TMA-2, the main metabolic step was not the *O*-demethylation but the hydroxylation of the 4-methyl moiety. So far, no studies have been published on the involvement of different cytochrome P450 (CYP) isoenzymes in this main metabolic step. However,

* Corresponding author. Tel.: +49 68411626050; fax: +49 68411626051.
E-mail address: hans.maurer@uniklinikum-saarland.de (H.H. Maurer).

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such studies are the first step for predicting possible risks of pharmacokinetic variations caused by pharmacogenetic variability or interactions with co-administered drugs or food ingredients [12,13]. They are routinely performed for substances intended for therapeutic use before registration, but not for drugs of the illicit market.

In clinical and forensic toxicology, drugs of abuse must be analyzed for monitoring an abuse or a poisoning. Several studies have been published on the detection of DOM itself in blood, urine, or brain tissue [10,11,14–16]. However, as shown by Ho et al., DOM itself was excreted into urine only to a very small extent, whereas the hydroxy metabolite played the main role in the excretion process [11]. Hence, this metabolite should be the target analyte for toxicological screening analysis in urine. This is in accordance to other compounds of this amphetamine class which could also be detected via their metabolites by the authors' systematic toxicological analysis (STA) [17–20].

The first aim of this study was to investigate the involvement of CYP isoenzymes in the formation of the main metabolite in a so-called initial activity screening. The second aim was the proof of a DOM intake by detection of the major metabolite as target analyte within the authors' STA procedure, which allows simultaneous detection of about 2000 other drugs, poisons and/or their metabolites in urine by GC–MS including many other amphetamine-derived drugs [17–20].

2. Experimental

2.1. Chemicals and reagents

DOM was provided by the Bundeskriminalamt (Wiesbaden, Germany) for research purposes. $NADP^+$ was obtained from Biomol (Hamburg, Germany), isocitrate and isocitrate dehydrogenase from Sigma (Taufkirchen, Germany), all other chemicals and reagents from Merck (Darmstadt, Germany). The following microsomes were from Gentest and delivered by NatuTec (Frankfurt/Main, Germany): baculovirus-infected insect cell microsomes (ICM, Supersomes®) containing 1 nmol/ml human cDNA-expressed CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, or 2 nmol/ml CYP2E1, wild-type baculovirus-infected ICM (control Supersomes®). Upon arrival, the microsomes were thawed at 37 °C, aliquoted, shock-frozen in liquid nitrogen and stored at −80 °C until use.

2.2. Microsomal incubations and workup for initial activity screening

The incubations were performed according to Refs. [21–24]. The mixtures (final volume: 50 μl, $n = 2$) consisted of 90 mM phosphate buffer (pH 7.4; for CYP1A2, CYP2B6, CYP2C8, CYP2C19, CYP2D6, CYP2E1, and CYP3A4) or Tris buffer (pH 7.4; for CYP2A6 and CYP2C9), 5 mM Mg^{2+}, 5 mM isocitrate, 1.2 mM $NADP^+$, 0.5 U/ml isocitrate dehydrogenase, 200 U/ml superoxide dismutase, 50 pmol/ml of CYP, and substrate at 37 °C. The substrates were added after dilution of 250 mM methanolic stock solutions in respective buffer. In none of the samples, the methanol concentration exceeded 0.4%. Reactions were started by adding the ice-cold microsomes and terminated with 5 μl of 60% (w/w) aqueous $HClO_4$. After termination, the samples were extracted by liquid–liquid extraction with 1 ml of dichloromethane–isopropanol–ethyl acetate (1:1:3; v/v/v) at pH 8–9. The organic layer was transferred into glass flasks and evaporated under reduced pressure at 70 °C to dryness, acetylated with 20 μl of an acetic anhydride–pyridine mixture (3:2; v/v) for 2 min under microwave irradiation at about 440 W. After evaporation of the derivatization mixture under reduced pressure at 70 °C, the residue was dissolved in 20 μl of methanol and 2 μl were injected into the GC–MS system.

2.3. Urine samples

The investigations were performed using urine of male Wistar rats (about 1 year old and 400 g body mass (BM), Ch. River, Sulzfleck, Germany) for toxicological diagnostic reasons according to the corresponding German law. They were administered a single 0.1 mg/kg BM as low dose and 10 mg/kg BM as high dose for the STA studies in aqueous suspension by gastric intubation. Urine was collected separately from the faeces over a 24 h period. The samples were immediately analyzed. Blank rat urine samples were collected before drug administration to check whether they were free of interfering compounds.

2.4. Sample preparation for toxicological analysis

A 5-ml portion of urine was worked-up as previously described for 4-MTA [25]. After acidic hydrolysis, the liquid–liquid extract was derivatized by acetylation. Aliquots (2 μl) were injected into the GC–MS system.

2.5. GC–MS apparatus and method

A Hewlett Packard (Agilent, Waldbronn, Germany) 5890 Series II gas chromatograph combined with a HP 5972A MSD mass spectrometer was used under the conditions described for 4-MTA [25]. The GC conditions were as follows: splitless injection mode; column, HP-1 capillary (12 m × 0.2 mm I.D.), cross-linked methyl silicone, 330 nm film thickness; injection port temperature, 280 °C; carrier gas, helium; flow-rate

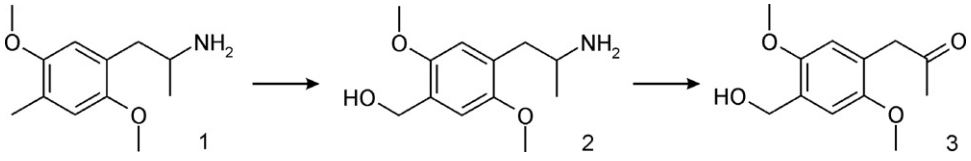

Fig. 1. Main metabolic steps of DOM in rats [11].

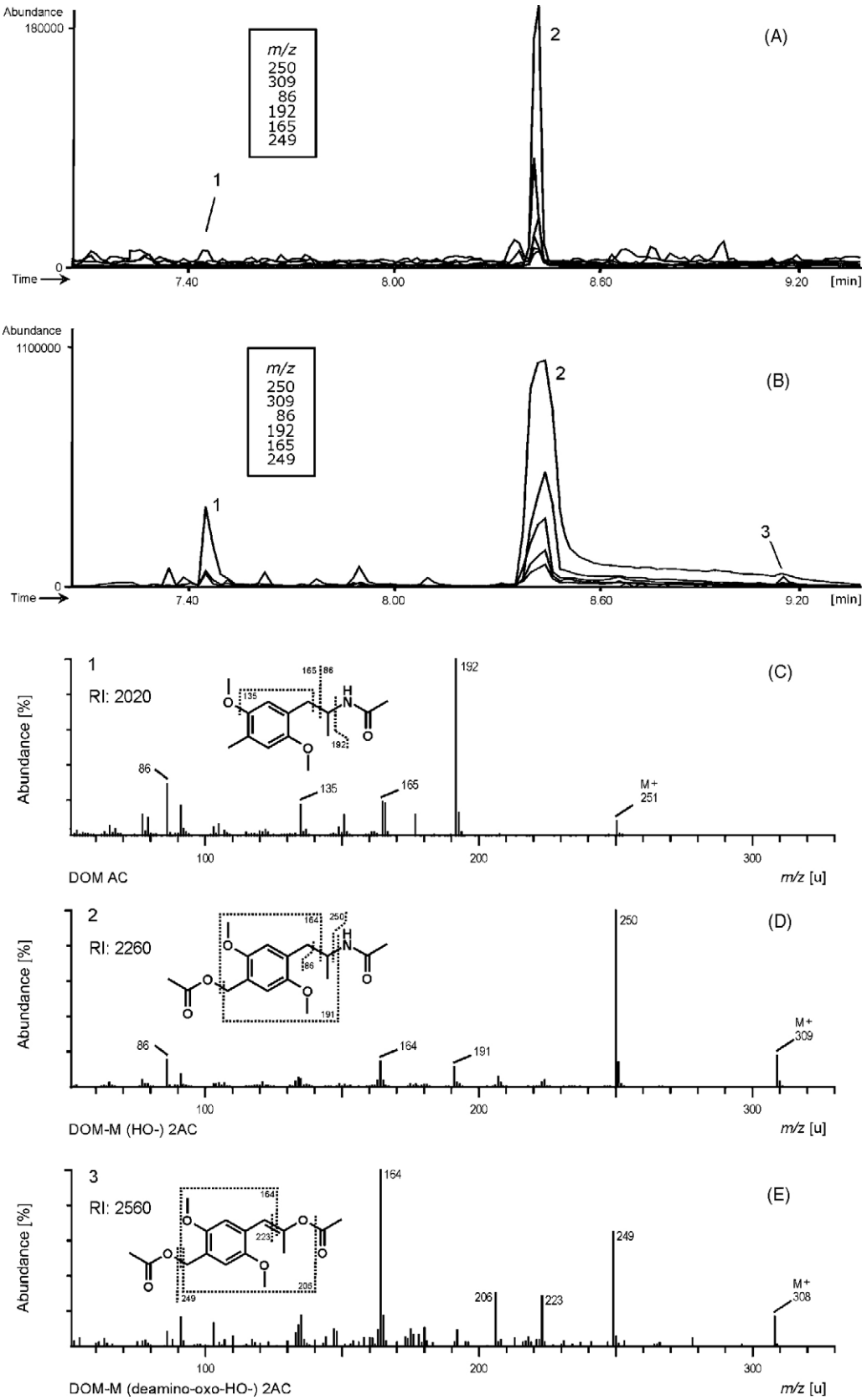

Fig. 2. Typical reconstructed mass chromatograms with the given ions of an acetylated extract of a rat urine sample collected over 24 h after intake of 0.1 (A) and 10 mg/kg BM of DOM (B). They indicate the presence of DOM (peak 1) and its main metabolite hydroxy DOM (peak 2) after administration of the low dose (A) and in addition the deamino-oxo-hydroxy metabolite (peak 3) after administration of the high dose (B). EI mass spectra, RIs, structures and predominant fragmentation patterns of DOM (C), its main metabolite hydroxy DOM (D) and the deamino-oxo-hydroxy metabolite (E) included in the STA procedure after acetylation. The numbers of the spectra correspond to those of the peaks in the upper part.

1 ml/min; column temperature, programmed from 100 to 310 °C at 30°/min, initial time 3 min, final time 8 min. The MS conditions were as follows: EI mode, ionization energy, 70 eV; ion source temperature, 220 °C; capillary direct interface, heated at 280 °C. For the initial activity screening, selected-ion monitoring using fragments *m/z* 250 and 309 for bis-acetylated hydroxy DOM and for STA, full-scan mode, *m/z* 50–800 u were used.

For toxicological detection of acetylated DOM and its main metabolites, mass chromatography with the selected ions *m/z* 250, 309, 86, 192, 165, and 249 was used. The identity of the peaks in the mass chromatograms was confirmed by computerized comparison of the mass spectra underlying the peaks (after background subtraction) with reference spectra recorded during previous metabolism studies [20,26].

3. Results and discussion

In Fig. 1, the main metabolic steps of DOM in rats are shown [11]. The initial activity screening for involvement of CYP isoenzyme in formation of the main metabolite, hydroxy DOM, showed that among the nine tested CYP isoenzymes, only CYP2D6 was capable to catalyze this reaction. Data based on the peak areas of hydroxy DOM are given for evaluation of the principle involvement of the CYPs. As CYP2D6 is polymorphically expressed and can be inhibited by co-administered drugs, the formation of hydroxy DOM might vary and affect its detectability in urine. DOM and/or other metabolites might be the target analyte in urine of CYP2D6 poor metabolizers.

Using the authors' STA procedure, the acetylated hydroxy metabolite was found to be the target analyte after low and high doses. It could be detected by mass chromatography with the ions *m/z* 250, 309, and 86. Fig. 2 shows typical reconstructed mass chromatograms with the given ions of an acetylated extract of a rat urine sample collected over 24 h after application of 0.1 mg/kg BM of DOM (A) which corresponded to a common users' dose and after application of 10 mg/kg BM (B) for simulating a DOM overdose. The lower part of Fig. 2 shows the EI mass spectra, the retention indices (RI), the structures and the predominant fragmentation patterns of the acetylated parent compound (C), the target analyte hydroxy-DOM (D), and the deamino-oxo-hydroxy metabolite (E) detectable after administration of the high dose. The postulated structures of the (derivatized) metabolites of DOM were deduced from the fragments detected in the EI mass spectra which were interpreted in correlation to those of the parent compound according to the rules described by, e.g. McLafferty and Turecek [27] and Smith and Busch [28]. The carboxy metabolite described by Ho et al. [11] could not be detected by the STA procedure most probably because of the extraction pH of 8–9 at which the carboxyl group is deprotonated. Although interferences by biomolecules or other drugs cannot be entirely excluded, they are rather unlikely, because their mass spectra and/or their RIs should be different.

For lack of authentic human urine samples, a comparison of the metabolites found in rat and human urine after administration of DOM was not yet possible. However, in other studies, good correspondence has been reported for the metabolic pathways as well as for the detectability between rats and humans [17,29–32].

4. Conclusions

Assuming similar metabolism, the described STA procedure should be suitable for proof of an intake of DOM in human urine by detection of its hydroxy metabolite besides traces of DOM. In poisoning cases the deamino-oxo-hydroxy metabolite should be detectable in addition. However, DOM and/or other metabolites such as deamino-oxo-hydroxy DOM might be the target analyte(s) in urine of genetic or functional CYP2D6 poor metabolizers.

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