Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 855 (2007) 127–133
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Proof of a 1-(3-chlorophenyl)piperazine (mCPP) intake—Use as adulterant of cocaine resulting in drug–drug interactions?☆

Roland F. Staack*, Liane D. Paul, Dagmar Schmid, Gabriele Roider, Burkhard Rolf

Institute of Forensic Medicine, Ludwig Maximilians University, D-80337 Munich, Germany

Received 5 February 2007; accepted 14 May 2007
Available online 29 May 2007

Abstract

Since 2005, increasing numbers of seizures of the designer drug of abuse 1-(3-chlorophenyl)piperazine (mCPP) have been reported. This paper describes the unequivocal proof of a mCPP intake. Differentiation from the intake of its precursor drugs trazodone and nefazodone was performed by a systematic toxicological analysis (STA) procedure using full-scan GC–MS after acid hydrolysis, liquid–liquid extraction and microwave-assisted acetylation. The found mCPP/hydroxy-mCPP ratio indicated altered metabolism of this cytochrome (CYP) 2D6 catalyzed reaction compared to published studies using the same procedure. Although this might be ascribed to a poor metabolizer (PM) phenotype, genotyping revealed no PM genotype but indications for an intermediate metabolizer genotype. However, a PM phenotype could also be caused by drug–drug interactions with CYP2D6 inhibitors or substrates such as the co-consumed cocaine and diltiazem and/or diltiazem metabolites, respectively. In conclusion, the presented data indicate a possible relevance of CYP2D6 polymorphism and/or drug interactions to mCPP toxicokinetics, which is important for risk assessment of this new designer drug of abuse, in particular if it is used as adulterant of CYP2D6 substrates such as cocaine.
© 2007 Elsevier B.V. All rights reserved.

Keywords: 1-(3-Chlorophenyl)piperazine; mCPP; Drug–drug interaction; Drug metabolism; GC–MS

1. Introduction

Besides Cannabis, synthetic drugs are the second most commonly used group of drugs of abuse in Europe [1]. So-called "piperazines" have been proffered as an alternative to amphetamine derived drugs of abuse since several years. This newer class of designer drugs of abuse includes benzylpiperazines, such as N-benzylpiperazine (BZP) itself, its methylenedioxy analogue 1-(3,4-methylenedioxybenzyl)piperazine (MDBP) and phenylpiperazines, e.g. 1-(3-trifluoromethylphenyl)piperazine (TFMPP), 1-(3-chlorophenyl)piperazine (mCPP) and 1-(4-methoxyphenyl)piperazine (MeOPP). Their abuse spread rapidly over the whole world, with BZP and TFMPP being the most abused compounds so far. Serious toxic effects as well as fatal poisonings have been reported [2–4]. The legal status of the piperazines is very inconsistent. As for mCPP, the discussion on its legal status has just begun and some European countries are considering control measures. Greece, Finnland and for a short time (March 2007) also Germany already control mCPP under drug control or equivalent legislation [5].

Since 2005, increasing numbers of seizures of mCPP have been reported from many countries [5]. mCPP is the most extensively pharmacologically characterized compound of the piperazines. Serotonin release by a serotonin transporter (SERT)-dependent mechanism [6–10], agonistic as well as antagonistic interactions with different serotonin receptors [11–13], inhibition of serotonin reuptake [8], slight dopamine release [9], as well as interactions with adrenergic and dopaminergic receptors [14] have been reported. Furthermore, mCPP has been extensively used in clinical psychiatry as a probe of serotonin function providing data on its pharmacological effects in humans [15–18].

The reason for mCPP abuse might be ascribed to the reported ethanol-like euphorogenic effects in abstinent alcoholics, "High" feelings as well as stimulant and hallucinogenic effects similar to those observed after LSD, mescaline or MDMA [19–27].

☆ This paper was presented at the 44th Scientific Meeting of The International Association of Forensic Toxicologists (TIAFT), Ljubljana, Slovenia, 27 August–1 September 2006.

* Corresponding author. Tel.: +49 89 5160 5111; fax: +49 89 5160 5144.
E-mail address: rstaack@gmx.de (R.F. Staack).

1570-0232/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2007.05.017

Despite the increasing number of mCPP seizures, only very scarce reports on the detection of mCPP in human body fluids can be found [5,28]. This paper describes the unequivocal proof of a mCPP intake. Possible pitfalls in toxicological analysis due to mCPP precursor drugs will be discussed as well as the found indications of the influence of the CYP2D6 phenotype and possible drug–drug interactions to mCPP pharmacokinetic.

2. Experimental

2.1. Chemicals and reagents

mCPP HCl was obtained from Sigma, Taufkirchen (Germany), trazodone was obtained from Dr. Karl Thomae GmbH (Biberach, Germany). All other chemicals and biochemicals were obtained from E. Merck (Darmstadt, Germany) and were of analytical grade. The oligonucleotide primers for multiplex PCR were obtained from MWG Biotech (Ebersberg, Germany), for real time PCR from Applied Biosystems (Foster City, USA).

2.2. Case history/blood and urine samples

Authentic blood and urine sample of a 29-year-old female had been submitted to our laboratory for toxicological analysis in the course of a criminal case. The proband admitted the consumption of cocaine, alcoholic drinks and acetaminophen. The blood and urine specimens were taken about 5.5 h after the last consumption of alcohol and cocaine. Acetaminophen had been consumed about 2 days before. The proband complained about feeling cold, dizziness and headache.

2.3. Analysis of the urine specimen

The urine specimen had been analyzed by immunoassay screening, by HPLC-UV analysis using the REMEDI^{TM} HS Drug Profiling System (Bio-Rad Laboratories, München, Germany) and by a GC–MS based systematic toxicological analysis (STA) procedure [29–31].

Immunoassay screening was performed on a Roche/Hitachi 912 automatic analyzer (Roche Diagnostics, Mannheim, Germany) using the Microgenics CEDIA or DRI assay for amphetamines (cut off 200 ng/mL), barbiturates (500 ng/mL), benzodiazepines (50 ng/mL), buprenorphine (5 ng/mL), cannabinoids (25 ng/mL), cocaine (100 ng/mL), EDDP (150 ng/mL), opiates (200 ng/mL), LSD (0.5 ng/mL), tricyclic antidepressants (100 ng/mL) (Microgenics Corp., Passau, Germany).

HPLC-UV analysis was performed on the REMEDI^{TM} HS Drug Profiling System. This automated analytical procedure was performed according to the manufacturer's recommendations, using 1 mL of urine.

Sample preparation for the GC–MS based STA procedure was according to Refs. [30,31]: the urine sample (5 mL) was divided into two equal aliquots. One aliquot was refluxed with 1 mL of 37% hydrochloric acid for 15 min. Following hydrolysis, the sample was mixed with 2 mL of 2.3 mol/L aqueous ammonium sulfate and 1.5 mL of 10 mol/L aqueous sodium hydroxide to obtain a pH value of 8–9. Before extraction, the aliquot of unhydrolyzed urine was added and this solution was extracted with 5 mL of a dichloromethane–isopropanol–ethyl acetate mixture (1:1:3, v/v/v). After phase separation by centrifugation, the organic layer was transferred and carefully evaporated to dryness. The residue was derivatized by acetylation with 100 μL of an acetic anhydride–pyridine mixture (3:2; v/v) for 5 min under microwave irradiation at about 440 W. After evaporation of the derivatization mixture, the residue was dissolved in 100 μL of methanol and 2 μL of this sample were injected into the GC–MS.

2.4. Gas chromatography–mass spectrometry

The extracts were analyzed using a Hewlett-Packard (Agilent, Waldbronn, Germany) 6890N gas chromatograph combined with a HP 5973 MSD mass spectrometer and a HP MS ChemStation (DOS series) with HP G1034D software version G1701 D01.02.16. The GC conditions were as follows: splitless injection mode; splitless time 2 min; column, Varian VF-5 ms capillary (30 m × 0.25 mm i.d.), cross-linked methyl silicone, 0.25 μm film thickness; injection port temperature, 280 °C; carrier gas, helium; flow-rate 1 mL/min; column temperature, programmed from 100 to 310 °C at 30 °C min^{-1}, initial time 2 min, final time 12 min. The MS conditions were as follows: full-scan mode, *m/z* 50–550; EI mode, ionization energy, 70 eV; ion source temperature, 230 °C; capillary direct interface, heated at 280 °C.

The full-scan GC–MS screening is based on reconstructed mass chromatography using macros for selection of suspected drugs followed by identification of the unknown spectra by library search using the Pfleger Maurer Weber (PMW) library as described in Refs. [29,30]. For toxicological analysis of mCPP and its metabolites, mass chromatography with the selected ions *m/z* 143, 145, 166, 182, 238, and 254 was used as described in Ref. [31].

2.5. Analysis of the plasma specimen

The plasma sample was screened for xenobiotics by using HPLC-DAD. Sample preparation for the HPLC-DAD was as follows: After addition of 3 μL methylclonazepam (0.1 mg/mL) as internal standard and dilution with 1 mL phosphate buffer pH 7.4, 1 mL of plasma was extracted with 5 mL 1-chlorbutan for 1 min on a Heidolph reax 2000 shaker (Schwabach, Germany). After phase separation by centrifugation (3 min at 5000 rpm) the organic layer was transferred into a glass vial and gently evaporated at 50 °C under a stream of nitrogen. The residue was reconstituted in 70 μL acetonitril:water (50:50, w/w) and transferred into an autosampler vial. Thirty microliters of this solution were injected into the HPLC-DAD system.

Furthermore, besides routine determination of blood alcohol concentration, confirmation analysis for cocaine and its metabolites by GC–MS after solid phase extraction of plasma followed by silylation was performed.

2.6. HPLC-DAD

The plasma sample was analyzed by HPLC-DAD. The system consisted of a Merck Hitachi L-7200 autosampler (Merck, Darmstadt, Germany), a Beckman M 114 pump (Beckman Instruments, San Remo, USA), a Waters Col.Htr.Mod. TCM column oven (Waters GmbH, Eschborn, Germany), a Hewlett-Packard (HP) diode array Series 110 (Hewlett-Packard, Waldbronn, Deutschland) recording between 190 and 400 nm with HP Chemstation Software (Revision A.10.02). Isocratic elution was performed on a Merck LiChroCARTcolumn (250 mm × 5 mm i.d.) with LiChrospher 60 RP Select B (50 μm, 60 A). The mobile phase consisted of acetonitrile and 0.02 M phosphate butter pH 2 (36:64, w/w) at a flow rate of 0.9 mL/min at 30 °C. Before use, the mobile phases were degassed for 30 min in an ultrasonic bath. Peak identification was performed by library search using the spectra library published by Pragst et al. [32].

2.7. CYP2D6 genotyping

Genomic DNA from whole blood was isolated using phenol chloroform extraction. Nucleotide numbering was consistent with the system recommended by the Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee (http://www.imm.ki.se/CYPalleles/).

The main mutations of the alleles CYP2D6*3 (2549A>del), *4 (1846G>A), *6 (1707T>del) and the mutation −1584C>G were identified using a multiplex PCR. The sequence and concentration of each primer in the PCR used in this study are listed in Table 1. The PCR was carried out in a total volume of 25 μL in the presence of 80 μM of each dNTP, 0.7 mM $MgCl_2$, 1 ng of genomic DNA as template and 1.25 U Ampli Taq Gold™ polymerase (Applied Biosystems Foster City, USA). After initial denaturation at 94 °C for 12 min, 35 cycles of 30 s at 94 °C, 45 s at 68 °C and 20 s at 72 °C were carried out. The final elongation step was at 60 °C for 60 min. The PCR products were examined by capillary electrophoresis with the ABI PRISM® 310 Genetic Analyzer (Applied Biosystems, Foster City, USA).

The complete deletion (Allele CYP2D6*5) or duplication of CYP2D6 was identified using TaqMan Real-Time PCR. The Quantifiler™ Human DNA Quantification Kit (Applied Biosystems, Foster City, USA) was used to detect the amount of human DNA. The quantification was carried out according to the manufacturer's instructions. The sequences of the primers and probe to detect the amount of CYP2D6 used in this study are shown in Table 2 and were used as described previously by Schaeffeler et al. [33]. Primers and probe were obtained from Applied Biosystems (Foster City, CA). Real-time PCR was performed using the ABI Prism 7300 sequence detection system.

Table 2
Sequences of primers and the probe for CYP2D6

Target	Name	Sequence and fluorescence label of the probe
CYP2D6	2D6 ex9 f3	5′-CTTCAC CTC CCTGCTGCAG-3′
	2D6 ex9 r3	5′-TCACCAGGA AAG CAA AGACA-3′
	2D6 ex9 probe	5′-FAM-CCG GCC CAG CCACCATGG-TAMRA

Amplification reactions (25 μL) to detect the amount of CYP2D6 were carried out in duplicate. 1 × TaqMan Universal Master Mix buffer (Applied Biosystems, Foster City, CA), 300 nM of each primer and 200 nM of the fluorogenic probe were used. Thermal cycling was initiated with a first denaturation step of 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and of 1 min at 60 °C. The size of the PCR product for CYP2D6 was 89 bp. In each experiment, a standard curve was recorded and a no-DNA control was included.

3. Results

3.1. Analysis of the urine specimen

The immunoassay screening was negative for amphetamines, barbiturates, benzodiazepines, buprenorphine, cannabinoids, EDDP, opiates, LSD, tricyclic antidepressants and positive for cocaine (for cut-off levels see Section 2.3).

The REMEDI™ HS Drug Profiling System indicated the following analytes: benzoylecgonine, cocaine or cocaethylene, mCPP.

By means of the GC–MS based STA procedure the following analytes could be detected: mCPP, HO-mCPP, both indicated as trazodone metabolites by the PMW library, diltiazem, O-demethyl diltiazem, deamino-hydroxy diltiazem, O-demethyl-deamino-hydroxy diltiazem, cocaine, cocaethylene, norcocaethylene and acetaminophen.

Table 1
Primer used for the multiplex PCR

Primer	Primer sequence (5′ → 3′)	Label	Concentration	Allele
1707Tdelf	5′-GTG GAT GGT GGG GCT AAT GCC TT-3′ [64]	6-FAM	0.275 μM	CYP2D6*6
1707Tdelr	5′-GCT TTG TGC CCT TCT GCC CAT CA-3′		0.275 μM	
2549Adelf	5′-TGA CCC AGC TGG ATG AGC TGC T-3′	HEX	0.175 μM	CYP2D6*3
2549Adelr	5′-CAT ACT CGG GAC AGA ACG GGG T-3′		0.175 μM	CYP2D6*9
1846GAwt	5′-TTA CCC GCA TCT CCC ACC CCC AG-3′	6-FAM	0.1 μM	
1846GAmut	5′-TTA CCC GCA TCT CCC ACC CCC AA-3′	HEX	0.125 μM	CYP2D6*4
1846GAr	5′-CAG AGA CTC CTC GGT CTC TCG CT-3′ [64]		0.1 μM	
−1584CGf	5′-GCA GCT GCC ATA CAA TCC ACC TG-3′		0.3 μM	
−1584wtr	5′-CCA GCT AAT TTT GTA TTT TTT GTA GAC ACC G-3′	6-FAM	0.4 μM	
−1584mutr	5′-CCA GCT AAT TTT GTA TTT TTT GTA GAC ACC G-3′	ROX	0.3 μM	

3.2. Analysis of the plasma specimen

By means of HPLC-DAD, mCPP could be detected in plasma. Routine measurement of blood alcohol concentration yielded a concentration of 0.87 g/L. The confirmation analysis for cocaine and its metabolites revealed the following compounds: benzoylecgonine (476 μg/L), ecgoninemethylester (56 μg/L), ecgoninethylester (45 μg/L).

3.3. CYP2D6 genotyping

The individual had one allele CYP2D6*5 (complete deletion of the CYP2D6 gene) and one functional allele, without any of the main mutations of the common nullalleles in the European population [34,35]. The functional allele however showed the wild type sequence −1584C in the promotor region which could be an advice for the allele CYP2D6*41 [33]. This allele encodes for an enzyme with decreased activity. This would mean that the individual showed the genotype CYP2D6*5/*41 and would be consequently an intermediate metabolizer (IM).

4. Discussion

Despite an increasing number of mCPP seizures, only very scarce reports on the detection on mCPP in human body fluids can be found. Only the report on mCPP by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) mentioned a few cases, in which mCPP had been detected in human body fluids, but without giving any details [5]. One possible explanation for the small number of reports might be that mCPP seems not to be detectable with common immunoassays, e.g. for amphetamines, which were often used for screening of specimens in clinical and forensic toxicology.

However, an intake of mCPP in human urine should be detectable by the STA procedure using full-scan GC–MS after acid hydrolysis, liquid–liquid extraction, and microwave assisted acetylation developed by Maurer et al. [29,36], as could be shown by a previous study conducted in rats [31]. As described in this study, mass chromatography with the ions *m/z* 143, 145, 166, 182, 238, and 254 indicated the presence of mCPP and/or its metabolites. Fig. 1 shows the corresponding reconstructed mass chromatograms of the analyzed urine specimen and the mass spectra underlying the peaks indicating the presence of mCPP and its metabolite HO-mCPP. Identity of the compounds was confirmed by comparison of the spectra with reference spectra of the PMW library [30]. However, detection of mCPP and its metabolites does not unequivocally prove the intake of mCPP. mCPP is also a metabolite of therapeutics such as trazodone and nefazodone.

An intake of mCPP can be differentiated from an intake of its precursor drugs in a urine sample by screening for the parent compounds or unique metabolites as described in Ref. [31]. No data is given whether such a differentiation was performed in those cases of mCPP detection in human body fluids which were mentioned in the EMCDDA report [5]. Differentiation between a mCPP intake and an intake of nefazodone is straightforward, as the unique nefazodone metabolites deamino hydroxy nefazodone and hydroxyethyl deamino hydroxy nefazodone should be found at higher abundances than mCPP and HO-mCPP [31]. These metabolites were not detected in the analyzed urine sample and consequently, a nefazodone intake could be ruled out. As reported, careful screening is necessary for differentiation between an intake of mCPP and trazodone, as only low abundant peaks of trazodone and its unique metabolite hydroxy trazodone are detectable in urine after intake of trazodone [31]. For exact determination of the trazodone retention time, trazodone reference substance was injected. Neither trazodone nor its unique metabolite were detected in the analyzed urine sample. That is why an intake of trazodone could also be ruled out.

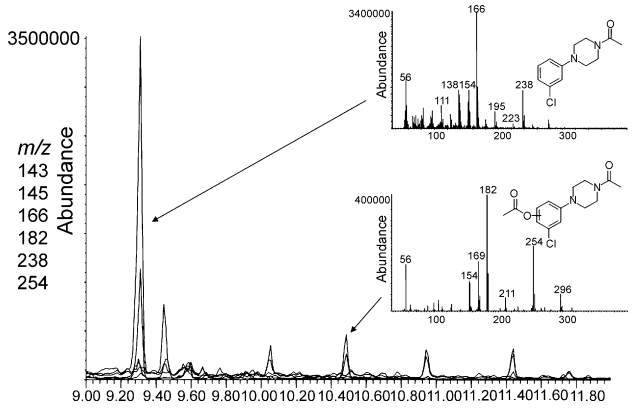

Fig. 1. Reconstructed mass chromatograms with the given ions of the acetylated extract of the urine sample, mass spectra underlying the indicated peaks and chemical structures of mCPP (upper spectrum) and HO-mCPP (lower spectrum) which were identified by library search.

The Remedi^{TM} HS drug profiling system is still widely used for urinalysis. The system only detected mCPP. HO-mCPP as well as acetaminophen and diltiazem were not detected. The Remedi^{TM} spectral database includes spectra of further trazodone metabolites, however the chemical structures of these compounds are not exactly specified, so it remains unclear, whether the system is able to detect HO-mCPP and the unique trazodone metabolites or not. Likewise, the structures of the listed nefazodone metabolites are not specified. Consequently, unequivocal differentiation between a mCPP intake and an intake of trazodone or nefazodone is not possible using the Remedi^{TM} system. However, this method also detected no trazodone or nefazodone metabolites besides mCPP in the case presented here.

The result of the urinalysis was further corroborated by the analysis of the corresponding plasma sample. Only mCPP could be detected. The identity of mCPP in the plasma sample was confirmed by comparison of the DAD spectrum and the retention time with those of reference substance (Fig. 2). The absence of trazodone was checked by analysis of a plasma sample spiked with a low therapeutic trazodone concentration of 0.3 mg/L. This concentration would have been detected by the used method. Furthermore, detection of mCPP at a higher concentration than trazodone after intake of trazodone is unlikely, as mCPP can only be found at minor concentrations after trazodone administration [37,38] and the elimination half-lives of trazodone (4.9 h, in young men) [37] and mCPP (4.2–4.7 h) are similar [18,39].

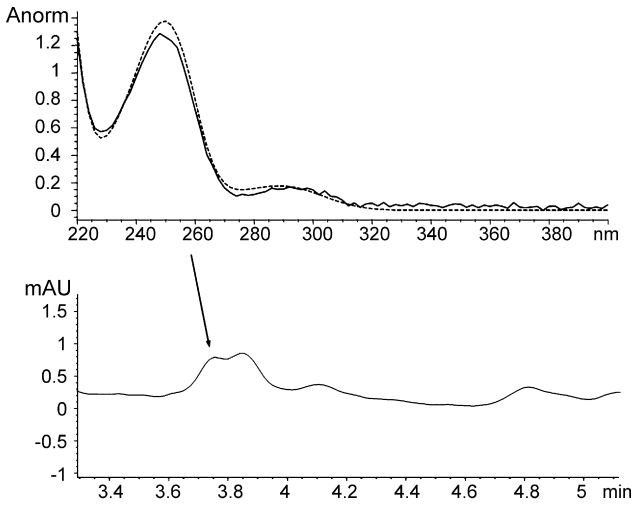

Fig. 2. HPLC-DAD chromatogram of the plasma sample (lower part), DAD spectrum of the indicated peak (upper part, solid line) and reference spectrum of mCPP (upper part, dotted line).

As a result of the performed analysis of the plasma and urine sample, the detected mCPP and HO-mCPP can unequivocally be ascribed to a mCPP intake.

As can be seen in Fig. 2, mCPP partly co-eluted with another compound which avoided exact quantification of mCPP. As only a very limited plasma volume was available, exact quantification, e.g. according to the method described in Ref. [40], was not possible. A very rough estimation suggested a concentration of about 16 μg/L.

In summary, the toxicological analysis revealed the intake of cocaine, acetaminophen, alcohol, mCPP and diltiazem. This result could explain the symptoms reported by the proband.

The consumption of cocaine, acetaminophen and alcohol was declared by the proband, whereas no information was given on any diltiazem or mCPP intake. A possible explanation might be that diltiazem is a known adulterant of cocaine with relative amounts up to 20% [41–44]. Likewise, mixtures of mCPP with cocaine have already been reported from the Netherlands and in some cases the mixtures were sold in form of powders as cocaine [5]. Thus, this raised the question, whether mCPP was mixed with the consumed cocaine. If so, the compounds were consumed nasally, as reported by the proband. There are no pharmacokinetic data for nasal application of mCPP. The reported bioavailabilities of 0.39 ± 0.3 [18] and 0.47 ± 0.29 [39] indicated a remarkable first pass effect of mCPP. Nasal application would bypass this first pass effect, which might result in elevated mCPP plasma concentrations compared to oral administration.

Interestingly, in this case, mCPP was the most abundant analyte in urine (Fig. 1). Considering the time difference between the reported consumption and the blood and urine sampling, this was astonishing and in contrast to the data of a previous study in male Wistar rats, a model of a CYP2D6 extensive metabolizer (EM) phenotype, which already proved good accordance with the corresponding results in humans [45,46]. In this study, HO-mCPP was the major analyte and the parent compound mCPP could only be detected at low abundance [31]. Furthermore, analysis of human urine after intake of the mCPP precursor drugs trazodone or nefazodone also confirmed HO-mCPP to be the major analyte detectable in urine [31,47]. In the study reported here and in the above mentioned studies, the same analytical procedure (GC–MS based STA procedure) was used [31]. Inversion of the drug/hydroxylated metabolite ratio in rat urine has been described for the structurally closely related compound 1-(3-trifluoromethylphenyl)-piperazine depending on the used model for the CYP2D6 phenotype [48]. Similar to the hydroxylation of TFMPP [49], the CYP dependent metabolism of mCPP to its major metabolite HO-mCPP has been reported to be catalyzed by CYP2D6 exclusively [50]. Accordingly, one might suggest that the proband in the case reported here showed a CYP2D6 poor metabolizer (PM) phenotype.

There is one study on mCPP pharmacokinetics in humans, which considered an influence of the CYP2D6 polymorphism as a possible explanation for variations of pharmacokinetic parameters [18]. The authors concluded that the elevated mCPP plasma concentrations could not be attributed to a deletion in the CYP2D6 gene. However, no explanation was given, whether the mentioned CYP2D6*5 deletion found in three of the probands were homozygote, i.e. real poor metabolizer, or heterozygot, resulting in CYP2D6 intermediate (IM) or extensive metabolizer genotype. Furthermore, they did not analyze the most common point mutations. This would be necessary to explain 93–98% of the PM in caucasians [34,35]. Thus, the role of CYP2D6 polymorphism in mCPP pharmacokinetic still remains unclear.

In order to clarify, whether the ratio of mCPP/HO-mCPP found in the current case might be explainable by a decreased metabolism due to a CYP2D6 PM genotype, CYP2D6 genotyping was performed. The individual showed the genotype CYP2D6*5/*41 and would consequently be an IM. The classification CYP2D6*41 is based on the wild type sequence −1584C in the promotor region and the absence of any frequent null allele mutations. Therefore, a further investigation of the 2988G>A mutation would give the final proof of the allele CYP2D6*41 [51]. Nevertheless, the CYP2D6 genotyping revealed that the proband did not show the CYP2D6 PM genotype caused by the most common nullallels [34,35].

A further possible explanation for the found mCPP/HO-mCPP ratio could be another well know phenomenon termed phenocopying, i.e. the change of the in vivo phenotype from EM to PM as a result of drug–drug interactions [52,53]. Inhibition of mCPP metabolism by CYP2D6 inhibitor quinidine could be shown in vitro [50]. Several in vivo studies performed with the mCPP precursor drug trazodone corroborated the susceptibility of mCPP concerning drug–drug interactions with other CYP2D6 substrates [54–56].

In the current case, besides mCPP, cocaine metabolites and diltiazem metabolites were detected. Cocaine is a known CYP2D6 inhibitor [57,58]. Furthermore, diltiazem and especially its metabolite deacetyl-diltiazem are CYP2D6 substrates [59,60]. Considering the fact that mCPP is metabolized by CYP2D6 exclusively [50], drug–drug interactions with the co-consumed compounds seem very likely. If the subject has a decreased CYP2D6 activity, as it is the case for an IM subject, it might be even more susceptible for drug–drug interactions.

In order to assess possible consequences of these findings, two things should be taken into consideration: Firstly, the fact that the occurrence of serotonin syndromes has been described after oral administration of mCPP at 0.5 mg/kg body weight [61], secondly, the fact that this dosage is close to the typically found mCPP doses in seizures, which are reported to be up to 46 mg [5]. Consequently, if mCPP plasma concentrations are increased due to genetic polymorphisms, drug–drug interactions or non-oral routes of administration, this might increase the risk of toxic side effects. As indicated by the reported case, special attention regarding toxicological risk assessment should be paid to mixtures of mCPP with other therapeutic drugs and/or drugs of abuse which are substrates or inhibitors of CYP2D6. Besides the combination of mCPP with cocaine, its combination with other CYP2D6 substrates such as methylenedioxymethamphetamine (MDMA) or the piperazines MeOPP and TFMPP has been described [5,48,62,63]. Whether drug interactions and/or genetic polymorphisms are really of clinical relevance for mCPP toxicokinetics, as indicated by the described case, cannot unequivocally be concluded yet. Further clinical data is needed in order to corroborate this assumption.

5. Conclusions

Here, we report on the detection of the designer drug mCPP in human body fluids. Unequivocal proof of a mCPP intake by differentiation from the intake of its precursor drugs trazodone and nefazodone was possible by the described GC–MS based STA procedure. The Remedi^{TM} HS drug profiling system did detect mCPP as a trazodone metabolite, but unequivocal differentiation from its precursor drugs was not possible by this method. Immunoassay screening for common drugs of abuse did not detect mCPP.

The found mCPP/HO-mCPP ratio indicated altered metabolism of this CYP2D6 catalyzed reaction, which might be explainable by a PM phenotype. By genotyping, no PM genotype could be detected, but indications for an IM genotype were found. Drug–drug interactions with co-consumed cocaine and diltiazem and/or diltiazem metabolites, known CYP2D6 inhibitors or substrates, respectively, could explain this finding. Furthermore, the detection of mCPP with cocaine and diltiazem, a known cocaine adulterant, point to a possible mixture of cocaine and mCPP, as recently reported from the Netherlands. Occurrence of serotonin symptoms after oral doses of mCPP similar to the commonly abused doses raises the question, whether CYP2D6 polymorphism and/or drug–drug interaction might lead to a higher toxic risk. For lack of sufficient human data, this question cannot unequivocally be answered yet. Thus, further clinical data is needed in order to corroborate this assumption.

Acknowledgements

The authors thank Inge Herrle, Gerlinde Schwarz, Agnes Pischinger, and Doris Harms for their support.

References

[1] European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), 2005.
[2] C. Balmelli, H. Kupferschmidt, K. Rentsch, M. Schneemann, Deutsche Medizinische Wochenschrift 126 (2001) 809.
[3] M. Wikstrom, P. Holmgren, J. Ahlner, J. Anal. Toxicol. 28 (2004) 67.
[4] P. Gee, S. Richardson, W. Woltersdorf, G. Moore, New Zealand Med. J. 118 (2005).
[5] European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), 2006.
[6] M.H. Baumann, D.C. Mash, J.K. Staley, Neuroreport 6 (1995) 2150.
[7] D.J. Pettibone, M. Williams, Biochem. Pharmacol. 33 (1984) 1531.
[8] M. Gobbi, M. Moia, L. Pirona, I. Ceglia, M. Reyes-Parada, C. Scorza, T. Mennini, J. Neurochem. 82 (2002) 1435.
[9] E. Eriksson, G. Engberg, O. Bing, H. Nissbrandt, Neuropsychopharmacology 20 (1999) 287.
[10] R.B. Rothman, M.H. Baumann, Pharmacol. Ther. 95 (2002) 73.
[11] D. Hoyer, D.E. Clarke, J.R. Fozard, P.R. Hartig, G.R. Martin, E.J. Mylecharane, P.R. Saxena, P.P. Humphrey, Pharmacol. Rev. 46 (1994) 157.
[12] P. Schoeffter, D. Hoyer, Naunyn Schmiedebergs Arch. Pharmacol. 339 (1989) 675.
[13] D. Fiorella, S. Helsley, R.A. Rabin, J.C. Winter, Psychopharmacol. Berl. 122 (1995) 237.
[14] A. Hamik, S.J. Peroutka, Biol. Psychiatry 25 (1989) 569.
[15] D.L. Murphy, K.P. Lesch, C.S. Aulakh, T.A. Pigott, Pharmacol. Rev. 43 (1991) 527.
[16] R.S. Kahn, S. Wetzler, Biol. Psychiatry 30 (1991) 1139.
[17] M.B. Gatch, Life Sci. 73 (2003) 1347.
[18] A. Feuchtl, M. Bagli, R. Stephan, C. Frahnert, H. Kolsch, K.U. Kuhn, M.L. Rao, Pharmacopsychiatry 37 (2004) 180.
[19] C. Benkelfat, D.L. Murphy, J.L. Hill, D.T. George, D. Nutt, M. Linnoila, Arch. Gen. Psychiatry 48 (1991) 383.
[20] E.A. Mueller, D.L. Murphy, T. Sunderland, J. Clin. Endocrinol. Metab. 61 (1985) 1179.
[21] D.L. Murphy, E.A. Mueller, J.L. Hill, T.J. Tolliver, F.M. Jacobsen, Psychopharmacol. Berl. 98 (1989) 275.
[22] J.H. Krystal, E. Webb, N. Cooney, H.R. Kranzler, D.S. Charney, Arch. Gen. Psychiatry 51 (1994) 898.
[23] E. Hollander, D.J. Stein, C.M. DeCaria, L. Cohen, J.B. Saoud, A.E. Skodol, D. Kellman, L. Rosnick, J.M. Oldham, Am. Psychiatry J. 151 (1994) 277.
[24] M.E. Tancer, C.E. Johanson, Drug Alcohol Depend. 65 (2001) 97.
[25] B.L. Buydens, M. Branchey, P. Fergeson, J. Hudson, C. McKernin, Biol. Psychiatry 41 (1997) 1071.
[26] B.L. Buydens, M. Branchey, P. Fergeson, J. Hudson, C. McKernin, Arch. Gen. Psychiatry 50 (1993) 1001.
[27] B.L. Buydens, M. Branchey, P. Fergeson, J. Hudson, C. McKernin, Alcohol Clin. Exp. Res. 21 (1997) 220.
[28] M.G. Bossong, J.P. Van Dijk, R.J.M. Niesink, Addict. Biol. 10 (2005) 321.
[29] H.H. Maurer, Clin. Chem. Lab Med. 42 (2004) 1310.
[30] K. Pfleger, H.H. Maurer, A. Weber, Mass Spectral Library of Drugs, Poisons, Pesticides, Pollutants and their Metabolites, Agilent Technologies, Palo Alto (CA), 2000.
[31] R.F. Staack, H.H. Maurer, J. Anal. Toxicol. 27 (2003) 560.
[32] F. Pragst, M. Herzler, S. Herre, B.T. Erxleben, M. Rothe, UV Spectra of Toxic Compounds, Helm-Verlag, Heppenheim (Germany), 2001.
[33] E. Schaeffeler, M. Schwab, M. Eichelbaum, U.M. Zanger, Hum. Mutat. 22 (2003) 476.
[34] C. Sachse, J. Brockmoller, M. Hildebrand, K. Muller, I. Roots, Pharmacogenetics 8 (1998) 181.
[35] A. Gaedigk, R.R. Gotschall, N.S. Forbes, S.D. Simon, G.L. Kearns, J.S. Leeder, Pharmacogenetics 9 (1999) 669.
[36] H.H. Maurer, in: K. Pfleger, H.H. Maurer, A. Weber (Eds.), Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and their Metabolites, Part 4, Wiley-VCH, Weinheim, 2000, p. 3.

[37] Boehringer Ingelheim Pharma GmbH & Co. KG in Rote Liste Sevice GmbH (Editor), FachInfo Fachinformationsverzeichnis, Rote Liste Sevice GmbH, Frankfurt/Main, 2004.
[38] M. Ishida, K. Otani, S. Kaneko, T. Ohkubo, T. Osanai, N. Yasui, K. Mihara, H. Higuchi, K. Sugawara, Int. Clin. Psychopharmacol. 10 (1995) 143.
[39] H.J. Gijsman, J.M. Van-Gerven, M.C. Tieleman, R.C. Schoemaker, M.S. Pieters, M.D. Ferrari, A.F. Cohen, G.M. Van-Kempen, J. Clin. Psychopharmacol. 18 (1998) 289.
[40] F.T. Peters, S. Schaefer, R.F. Staack, T. Kraemer, H.H. Maurer, J. Mass Spectrom. 38 (2003) 659.
[41] Bundeskriminalamt, Bundeslagebild Rauschgift 2004 Bundesrepublik Deutschland, 2005.
[42] Drug Enforcement Administration-Office of Forensic Sciences, Microgram Bull. 38 (2005) 2.
[43] Drug Enforcement Administration-Office of Forensic Sciences, Microgram Bull. 38 (2006) 181.
[44] D.E. Peters, Microgram J. 2 (2004) 11.
[45] R.F. Staack, G. Fritschi, H.H. Maurer, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 773 (2002) 35.
[46] R.F. Staack, H.H. Maurer, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 798 (2003) 333.
[47] J. Bickeboeller-Friedrich, H.H. Maurer, Ther. Drug Monit. 23 (2001) 61.
[48] R.F. Staack, L.D. Paul, D. Springer, T. Kraemer, H.H. Maurer, Biochem. Pharmacol. 67 (2004) 235.
[49] R.F. Staack, G. Fritschi, H.H. Maurer, J. Mass Spectrom. 38 (2003) 971.
[50] R.F. Staack, H.H. Maurer, Curr. Drug Metab. 6 (2005) 259.
[51] S. Raimundo, C. Toscano, K. Klein, J. Fischer, E.U. Griese, M. Eichelbaum, M. Schwab, U.M. Zanger, Clin. Pharmacol. Ther. 76 (2004) 128.
[52] U.M. Zanger, S. Raimundo, M. Eichelbaum, Naunyn Schmiedebergs Arch. Pharmacol. 369 (2004) 23.
[53] H.K. Kroemer, M. Eichelbaum, Life Sci. 56 (1995) 2285.
[54] M. Maes, H. Westenberg, E. Vandoolaeghe, P. Demedts, A. Wauters, H. Neels, H.Y. Meltzer, J. Clin. Psychopharmacol. 17 (1997) 358.
[55] N. Yasui, K. Otani, S. Kaneko, T. Ohkubo, T. Osanai, M. Ishida, K. Mihara, T. Kondo, K. Sugawara, Y. Fukushima, Ther. Drug Monit. 17 (1995) 333.
[56] K. Mihara, K. Otani, M. Ishida, N. Yasui, A. Suzuki, T. Ohkubo, T. Osanai, S. Kaneko, K. Sugawara, Ther. Drug Monit. 19 (1997) 43.
[57] Y. Ramamoorthy, A.M. Yu, N. Suh, R.L. Haining, R.F. Tyndale, E.M. Sellers, Biochem. Pharmacol. 63 (2002) 2111.
[58] R.F. Tyndale, R. Sunahara, T. Inaba, W. Kalow, F.J. Gonzalez, H.B. Niznik, Mol. Pharmacol. 40 (1991) 63.
[59] E. Molden, A. Asberg, H. Christensen, Eur. J. Clin. Pharmacol. 56 (2000) 575.
[60] E. Molden, A. Asberg, H. Christensen, Drug Metab. Dispos. 30 (2002) 1.
[61] T. Klaassen, K.L. Ho Pian, H.G. Westenberg, J.A. den Boer, H.M. van Praag, Psychiatry Res. 79 (1998) 207.
[62] T. Kraemer, H.H. Maurer, Ther. Drug Monit. 24 (2002) 277.
[63] R.F. Staack, D.S. Theobald, L.D. Paul, D. Springer, T. Kraemer, H.H. Maurer, Xenobiotica 34 (2004) 179.
[64] S.L. Wang, M.D. Lai, J.D. Huang, Drug Metab. Dispos. 27 (1999) 385.