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Metabolism and toxicological detection of the designer drug *N*-(1-phenylcyclohexyl)-3-methoxypropanamine (PCMPA) in rat urine using gas chromatography–mass spectrometry

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ABSTRACT

Studies on the metabolism and the toxicological detection of the phencyclidine-derived designer drug *N*-(1-phenylcyclohexyl)-3-methoxypropanamine (PCMPA) in rat urine are described using gas chromatographic–mass spectrometric (GC–MS) techniques. Based on the identified metabolites, the following metabolic pathways could be postulated: *N*-dealkylation, *O*-demethylation partially followed by oxidation of the resulting alcohol to the corresponding carboxylic acid, hydroxylation of the cyclohexyl ring at different positions, and aromatic hydroxylation. The formed metabolites were identical to those of the homologue *N*-(1-phenylcyclohexyl)-3-ethoxypropanamine (PCEPA) with exception of the mono hydroxyl metabolites of PCEPA. All PCMPA metabolites were partially excreted in conjugated form. An intake of a common drug users' dose of PCMPA could be detected in rat urine by the authors' systematic toxicological analysis (STA) procedure using full-scan GC–MS after acid hydrolysis, liquid–liquid extraction and microwave-assisted acetylation. The STA should be suitable for proof of an intake of PCMPA also in human urine assuming similar metabolism.

1. Introduction

Several new synthetic drugs from various classes were seized in the German federal state of Hesse and surrounding federal states in the late 1990s. Some of them were phencyclidine derivatives such as *N*-(1-phenylcyclohexyl)-propanamine (PCPR), *N*-(1-phenylcyclohexyl)-3-methoxypropanamine (PCMPA), *N*-(1-phenylcyclohexyl)-2-methoxyethanamine (PCMEA), and *N*-(1-phenylcyclohexyl)-2-ethoxyethanamine (PCEEA). The seized preparations contained either one compound or mixtures with other designer drugs [1]. So far, little information is available on pharmacological properties of these compounds. However, they might be assumed to be similar to those of the structurally related drugs phencyclidine and ketamine, which both act as antagonists at *N*-methyl-D-aspartate (NMDA) receptors and have psychotomimetic as well as anesthetic properties [2]. Furthermore, it has been reported that (1-phenylcyclohexyl)-amine produced a long-lasting dose-dependent effect on the efflux of dopamine in the rat [3]. This compound is a known metabolite of phencyclidine [3], PCPR [4], and the related compounds *N*-(1-phenylcyclohexyl)-3-ethoxypropanamine (PCEPA) [5], *N*-(1-phenylcyclohexyl)-2-ethoxyethanamine (PCEEA) and *N*-(1-phenylcyclohexyl)-2-methoxyethanamine (PCMEA) [6]. Certainly, the described pharmacological profiles of the phencyclidine derivatives would be in line with their abuse as designer drugs. Studies on the metabolism and toxicological detection of PCEPA, PCPR, PCEEA and PCMEA have been described [4–6], but not yet for PCMPA. Therefore, the aim of this study was to identify the metabolic pathways of PCMPA and to elucidate the detectability of PCMPA and its metabolites within the authors' systematic toxicological analysis (STA) procedure in urine. This procedure is a widely used very comprehensive screening procedure covering thousands of drugs, poisons and/or their metabolites [7–10].

2. Experimental

2.1. Chemicals and reagents

PCMPA·HCl was provided by the Hessisches Landeskriminalamt (Wiesbaden, Germany) for research purposes. The purity was checked by gas chromatographic–mass spectrometry (GC–MS) and HPLC. *N*-Methyl-bis-trifluoroacetamide (MBTFA)

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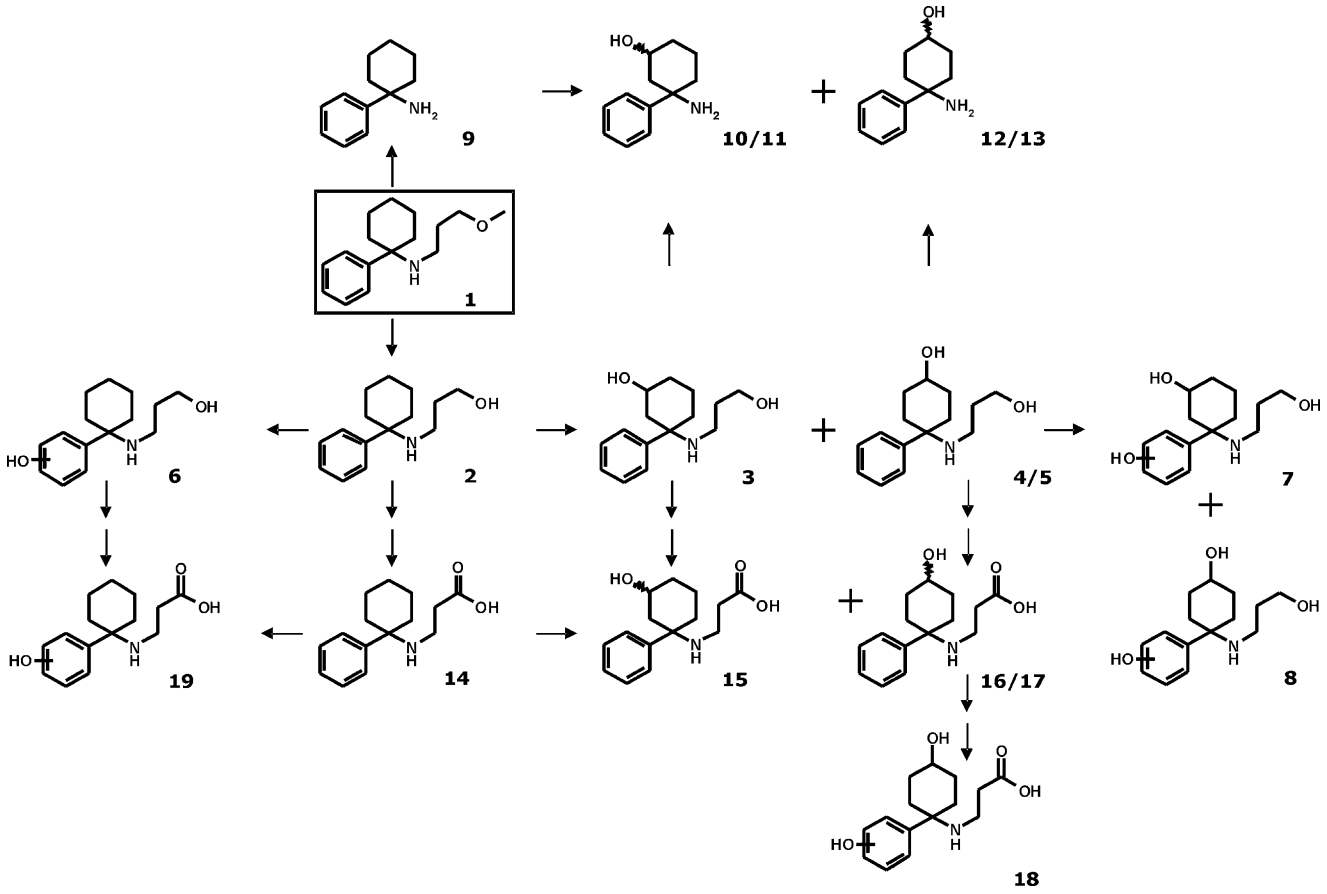

Fig. 1. Metabolic pathways of PCMPA.

and *N*-methyl-*N*-trimethylsilyl trifluoroacetamide (MSTFA) were obtained from Fluka (Taufkirchen, Germany). Bond Elut Certify cartridges (130 mg, 3 ml) were obtained from Varian (Darmstadt, Germany). All other chemicals and biochemicals were obtained from Merck (Darmstadt, Germany). All chemicals and biochemicals were of analytical grade.

2.2. Urine samples

Urine samples of male Wistar rats (Ch. River, Sulzfleck, Germany) were used in these studies for toxicological diagnostic reasons according to the corresponding German law. The rats received a single 20 mg/kg body mass (BM) dose of PCMPA for metabolism studies or a single 0.1 mg/kg BM dose for STA studies in aqueous suspension by gastric intubation ($n = 3$ each). Urine was collected separately from the faeces over a 24-h period at 20 °C stabilized with sodium fluoride. All samples were directly analyzed. Blank rat urine samples were collected before drug administration to check whether they were free of interfering compounds.

2.3. Sample preparation for metabolism studies

Aliquots of urine (3 ml) were worked-up as previously described for PCEPA [5]. After enzymatic cleavage of the conjugates using glucuronidase (EC no. 3.2.1.31) and arylsulfatase (EC no. 3.1.6.1) from *Helix pomatia*, the samples were extracted using Bond Elut Certify cartridges. The resulting extracts were derivatized by acetylation, trifluoroacetylation or trimethylsilylation and aliquots (2 μl) of the derivatized extracts were analyzed by GC–MS. The same procedures with the exception of enzymatic hydrolysis were used to study whether the metabolites were excreted as glucuronide and/or sulfate conjugates.

2.4. Sample preparation for STA

A 5-ml portion of urine was worked-up as described for other designer drugs [5,11]. After cleavage of conjugates by acid hydrolysis using hydrochloric acid and liquid–liquid extraction at pH 8–9 using a mixture of dichloromethane–

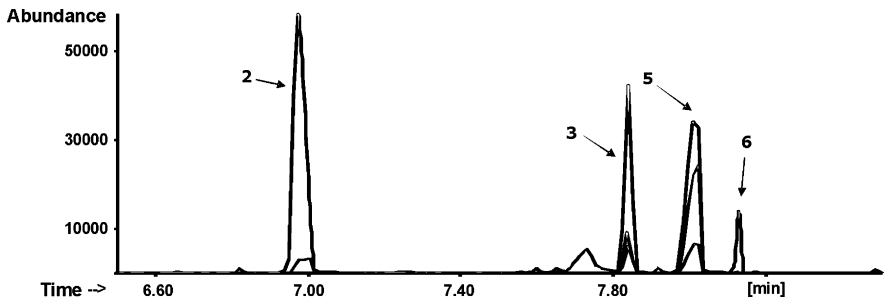

Fig. 2. Typical reconstructed mass chromatograms with ions *m*/*z* 232, 274, 273, and 290 of an acetylated extract of a rat urine sample collected over 24 h after application of 0.1 mg/kg BM of PCMPA which corresponded to a common drug users' dose of about 7 mg. The identity of peaks in the mass chromatograms was confirmed by computerized comparison of the underlying mass spectra with the reference spectra [9] (The numbers correspond to the respective metabolites in Fig. 1 and mass spectra in Fig. 3).

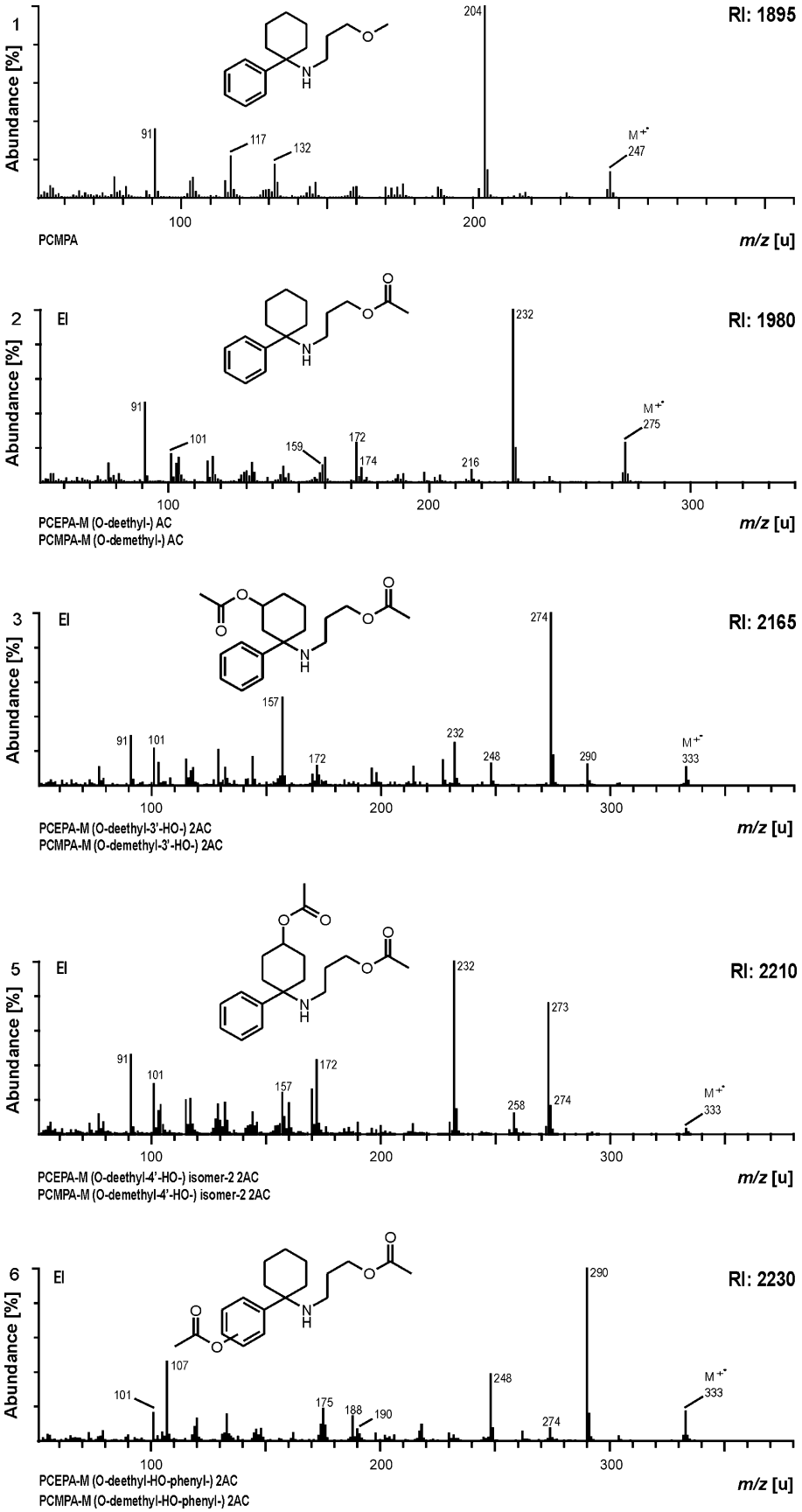

Fig. 3. Mass spectra, structures and RIs of PCMPA and metabolites after acetylation used as target analytes for STA procedure (the numbers correspond to the respective metabolites in Fig. 1).

isopropanol–ethyl acetate, the sample was derivatized by acetylation. Aliquots (2 μl) of the derivatized extract were injected into the GC–MS.

2.5. GC–MS apparatus for metabolism studies

The extracts were analyzed using a Hewlett Packard (HP, Agilent, Waldbronn, Germany) 5890 Series II gas chromatograph combined with an HP 5989B MS Engine mass spectrometer and an HP MS ChemStation (DOS series) with HP G1034C software version C03.00. 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 1 ml/min; column temperature, programmed from 100–310 °C at 30°/min, initial time 3 min, final time 8 min. The MS conditions were as follows: full-scan mode, *m*/*z* 50–800 u; electron ionization (EI) mode, ionization energy, 70 eV; ion source temperature, 220 °C; capillary direct interface, heated at 260 °C.

2.6. GC–MS apparatus for STA

The extracts were analyzed using a HP 5890 Series II gas chromatograph combined with a HP 5972A MSD mass spectrometer and a HP MS ChemStation (DOS series) with HP G1034C software version C03.00. 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 1 ml/min; column temperature, programmed from 100–310 °C at 30°/min, initial time 3 min, final time 8 min. The MS conditions were as follows: full-scan mode, *m*/*z* 50–550 u; EI mode, ionization energy, 70 eV; ion source temperature, 220 °C; capillary direct interface, heated at 280 °C.

2.7. GC–MS procedure for identification of metabolites and STA

PCMPA and its metabolites were separated by GC and identified by MS in the derivatized urine extracts. For toxicological detection of PCMPA and its metabolites, mass chromatography was used extracting characteristic fragment ions from the total ion current. The following ions were used for this purpose: *m*/*z* 232, 274, 273, and 290. These ions were selected from the mass spectra recorded during this study. 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 the recorded reference spectra [9].

3. Results and discussion

The postulated structures of the (derivatized) metabolites of PCMPA were deduced from the fragments detected in the EI mass spectra which were interpreted in correlation to those of the parent compound which was described by Roesner et al. [1] and according to the rules described by, e.g. McLafferty and Turecek [12] and Smith and Busch [13]. Details on the fragmentation patterns were have already been extensively discussed for the metabolites of the structural homologue PCEPA [5]. Furthermore, Peters et al. have biotechnological synthesized the common (*O*-dealkyl) metabolite of PCMPA and PCEPA and the structure confirmed by GC–MS and ^{1}H NMR [14].

In the acetylated urine extract, PCMPA and the acetyl derivatives of the following metabolites were identified. The numbers of the metabolites in Fig. 1 are given in brackets: PCMPA (1), *O*-demethyl PCMPA (2), *O*-demethyl-3′-hydroxy PCMPA (3), *O*-demethyl-4′-hydroxy PCMPA isomer 1 (4), *O*-demethyl-4′-hydroxy PCMPA isomer 2 (5), *O*-demethyl-hydroxyphenyl PCMPA (6), *O*-demethyl-3′-hydroxy-hydroxyphenyl PCMPA (7), *O*-demethyl-4′-hydroxy-hydroxyphenyl PCMPA (8), *N*-dealkyl PCMPA (9), *N*-dealkyl-3′-hydroxy PCMPA isomer 1 (10), *N*-dealkyl-3′-hydroxy-PCMPA isomer 2 (11), *N*-dealkyl-4′-hydroxy PCMPA isomer 1 (12), *N*-dealkyl-4′-hydroxy PCMPA isomer 2 (13).

In the trimethylsilylated urine extract, PCMPA and the trimethylsilyl derivatives of the following compounds could additionally be identified: carboxy PCMPA (14), carboxy-3′-hydroxy PCMPA (15), carboxy-4′-hydroxy PCMPA isomer 1 (16), carboxy-4′-hydroxy PCMPA isomer 2 (17), carboxy-4′-hydroxy-hydroxyphenyl PCMPA (18), carboxy-hydroxyphenyl PCMPA (19).

O-dealkylation and *N*-dealkylation of PCMPA lead to the same metabolites (2 and 9) as formed by PCEPA [5]. All downstream metabolites deriving from these were also identical for both drugs. Therefore, common metabolic pathways of PCMPA and PCEPA could be concluded with exception of mono hydroxylation of the parent drug, which was observed for PCEPA but not for PCMPA. The metabolic pathways for PCMPA are depicted in Fig. 1. As the peak areas after cleavage of conjugates have been higher than without, it can be concluded that the metabolites were partially excreted in conjugated form. Therefore, cleavage of conjugates prior to analysis can increase the detection window.

The authors' STA procedure allowed the detection of an intake of a dose of PCMPA that corresponds to a common drug users' dose in urine. Fig. 2 shows typical reconstructed mass chromatograms with the ions *m*/*z* 232, 274, 273, and 290 of an acetylated extract of a rat urine sample collected over 24 h after application of a single dose of 0.1 mg/kg BM of PCMPA which corresponded to a dose of about 7 mg corresponding to that of seized tablets containing other phencyclidine derivatives. They indicate *O*-demethyl PCMPA (2), *O*-demethyl-3′-hydroxy PCMPA (3), *O*-demethyl-4′-hydroxy PCMPA isomer 2 (5) and *O*-demethyl-hydroxyphenyl PCMPA (6). The respective reference spectra for identification via library search are given in Fig. 3. This can be done automatically using computerized comparison of the underlying mass spectrum with the corresponding reference library [9].

4. Conclusions

The presented metabolism studies showed that the designer drug PCMPA was mainly metabolized to the same metabolites as PCEPA. Therefore, a low dose intake of one or two of these drugs could not be differentiated. Only after the high doses, both could be differentiated by detection of the respective parent drugs or detection of mono hydroxylated PCEPA.

The authors' experience in metabolism and analytical studies on rats and humans support the assumption that the metabolites found in rat urine should also be present in human urine [15–19]. Therefore, it can be concluded that the procedure should also be applicable for human urine screening for PCMPA in clinical or forensic cases, however considering the possible problem of differentiation.

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