

Comparative Studies on the Metabolism and the Detection of Phencyclidine-derived Designer Drugs in Rat Urine Using GC-MS Techniques

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Abstract

The aim of this study was to compare these drugs with respect to their metabolites in rat urine and their detectability within our systematic toxicological analysis (STA) procedure. PCEEA and PCMEA formed identical metabolites. PCEPA and PCMPA also formed identical metabolites except the PCEPA mono-hydroxy metabolites. According to the identified metabolites, the following metabolic steps could be postulated for PCEPA, PCMPA, PCEEA, and PCMEA: N-dealkylation, O-deethylation followed by oxidation to the corresponding acid, hydroxylation of the cyclohexyl ring at different positions, aromatic hydroxylation, and finally combination of those. PCPR was found to be metabolized by partly overlapping N-dealkylation, hydroxylation at different positions of the cyclohexyl ring, the aromatic system, and the side chain. 1-Phenylcyclohexanamine could be identified as common metabolite of all studied drugs. After low dose application, all studied drugs were detectable by STA via their metabolites. The phencyclidine-derived designer drugs were extensively metabolized so that metabolites are the targets for urinalysis. Assuming similar metabolism in humans, the authors' STA should be suitable to prove an intake of any of the studied drugs in human urine.

1. Introduction

N-(1-Phenylcyclohexyl)-N-3-ethoxypropanamine (PCEPA), N-(1-Phenylcyclohexyl)-N-3-methoxypropanamine (PCMPA), N-(1-Phenylcyclohexyl)-N-2-ethoxyethanamine (PCEEA), N-(1-Phenylcyclohexyl)-N-2-methoxyethanamine (PCMEA), and N-(1-Phenylcyclohexyl)-N-propanamine (PCPR) are phencyclidine-derived designer drugs which were seized in the end of the 1990s in the German federal state of Hesse and the surrounding federal states [4]. So far, procedures for the detection of PCEPA and/or its metabolites have been described confirming the consumption of this designer drug [5]. The aim of this study was to compare these drugs with respect to their metabolites in rat urine and their detectability within our systematic toxicological analysis (STA) procedure.

2. Experimental

2.1 Chemicals and reagents

PCEPA HCl, PCMPA HCl, PCEEA HCl, PCMEA HCl, PCPR HCl were provided by the Hessisches Landeskriminalamt (Wiesbaden, Germany) for research purposes. Methyl-bis(trifluoroacetamide) (MBTFA) and *N*-methyl-*N*-trimethylsilyl trifluoroacetamide (MSTFA) were obtained from Fluka (Taufkirchen, Germany). Isolute Confirm HCX cartridges were obtained from Separtis (Grenzach-Wyhlen, Germany). Bond Elut Certify cartridges 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

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 each containing 1 nmol/ml human cDNA-expressed CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 (2 nmol/ml), or CYP3A4 (Supersomes[®]), wild-type baculovirus-infected insect cell microsomes (control Supersomes[®]), pooled human liver microsomes (pHLM 20 mg microsomal protein/ml, 400 pmol total CYP/mg protein) and single donor human liver microsomes with poor metabolizer genotype (PM HLM, 20 mg microsomal protein/ml). After delivery, the microsomes were thawed at 37°C, aliquoted, shock-frozen in liquid nitrogen and stored at -80°C until use.

2.2 Urine samples

The investigations were performed using urine of male rats (Wistar, Ch. River, Sulzleck, Germany) for toxicological diagnostic reasons according to the corresponding German law. They were administered a single 20 mg/kg body mass (BM) dose for metabolism studies or a 0.1 mg/kg BM dose for the STA study in aqueous suspension by gastric intubation (n = 2 for each dose). Urine was collected separately from the faeces over a 24 h period. Blank rat urine samples were collected before drug administration to check whether they were free of interfering compounds.

2.3 Sample preparation

The samples were worked up as described in reference [5]. Briefly, for metabolism studies, a 5-ml portion of urine was extracted by solid-phase extraction with or without prior enzymatic cleavage of conjugates followed by acetyla-

tion or trifluoroacetylation and/or trimethylsilylation. For STA, a 5-ml portion of urine was divided into two aliquots. One aliquot was refluxed with HCl. The combined portions were worked-up by liquid-liquid extraction and acetylation.

2.4 GC-MS analysis

The samples were analyzed using a Hewlett Packard (Agilent, Waldbronn, Germany) 5890 Series II GC combined with an HP 5989B MS Engine mass spectrometer and an HP MS ChemStation (DOS series) with HP G1034C software. The GC was equipped with an HP-1 capillary column and the MS was operated in the EI and PICI full scan mode. For details see reference [5, 5].

2.5 GC-MS procedure for identification of metabolites and STA by GC-MS

PCEPA, PCMPA, PCEEA, PCEMA, PCPR and its metabolites were separated by GC and identified by MS in acetylated urine extracts. For toxicological analysis mass chromatography was used extracting characteristic fragment ions of the PCPs and their metabolites from the total ion current. The following ions were used for this purpose: m/z 218, 259, 260 for PCEEA and its metabolites and m/z 232, 273, 274, 290 for PCEPA and its metabolites. The identity of the peaks in the mass chromatograms was confirmed by computerized comparison [2] of the mass spectra underlying the peaks (after background subtraction) with reference spectra recorded during this study [1, 2, 5].

2.6 Microsomal incubations

The microsomal incubations were performed as described in reference [7]. Briefly, mixtures (final volume: 50 μ l) consisted of 90 mM phosphate buffer (pH 7.4), 5 mM Mg^{2+} , 5 mM isocitrate, 1.2 mM NADP⁺, 0.5 U/ml isocitrate dehydrogenase, 200 U/ml superoxide dismutase and substrate at 37°C. The substrate was diluted in the above-mentioned phosphate buffer and added into the incubation mixture. Reactions were started by addition of ice-cold microsomes and terminated with 5 μ l of 60% (w/w) perchloric acid. Incubations were performed with 50 μ M PCEPA HCl, PCMPA HCl, PCEEA HCl, PCMEA HCl, PCPR HCl and 50 pmol/ml CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4 for 30 min. For incubations with CYP2A6 or CYP2C9, phosphate buffer was replaced with 45 mM or 90 mM Tris-buffer, respectively, according to the Gentest manual. The samples were worked up by solid phase extraction and after acetylation injected into the GC-MS [5].

3. Results and discussion

3.1. Identification of metabolites

The urinary metabolites of the PCPs were separated by GC and identified by EI MS and PICI MS. The postulated structures of the (derivatized) metabolites were deduced from the fragments detected in the EI mode which were interpreted in correlation to those of the parent compounds according to the rules described by e.g. McLafferty and Turecek [3] and Smith and Busch [6]. The EI and PICI mass spectra, the retention indices, the structures and predominant fragmentation patterns of PCEPA and its metabolites after derivatization are shown in reference [5]. However, using acetylation as derivatization procedure, physiologically acetylated metabolites cannot be differentiated from acetyl derivatives. For this particular question, the presence of acetylated metabolites was confirmed in urine extracts after trifluoroacetylation. For detection of acidic metabolites, the enzymatically cleaved urine samples were extracted at pH value 5.2 by solid phase extraction. Solid phase extraction could also be performed for acidic metabolites, as there exist a basic and an acidic center. Because of this property these compounds will be charged after washing with hydrochloric acid and in this way the ions are able to bind to the anion exchange resin. The extraction was followed by trimethylsilylation. This derivatization step was necessary as there was no alternative possibility to distinguish the molar masses of the basic metabolites from the acidic metabolites. Based on the identified compounds, the following metabolic pathways could be postulated for PCEPA, PCMPA, PCEEA, and PCMEA: N-dealkylation, O-deethylation followed by oxidation to the corresponding acid, hydroxylation of the cyclohexyl ring at different positions, aromatic hydroxylation, and finally combination of those. PCPR was found to be metabolized by partly overlapping N-dealkylation, hydroxylation at different positions of the cyclohexyl ring, the aromatic system, and the side chain. PCEPA and PCMPA formed the same main metabolite by O-dealkylation. In the following metabolic pathway both compounds also showed the identical metabolites with the exception that additionally PCEPA formed mono-hydroxylated isomers to a minor extent. Also PCEEA and PCMEA formed identical metabolites as also the same O-dealkylated main metabolite could be determined for both compounds. That means neither the propyl derivatives (PCEPA, PCMPA) nor the ethyl derivatives (PCEEA, PCMEA) could be differentiated among each other by their metabolites. In contrast, the propyl and ethyl derivatives could be differentiated by their metabolites.

1-Phenylcyclohexanamine and its hydroxyl isomers could be identified as common metabolites of all studied drugs. The common metabolic pathways of the main metabolites of PCPs are depicted in Fig. 1.

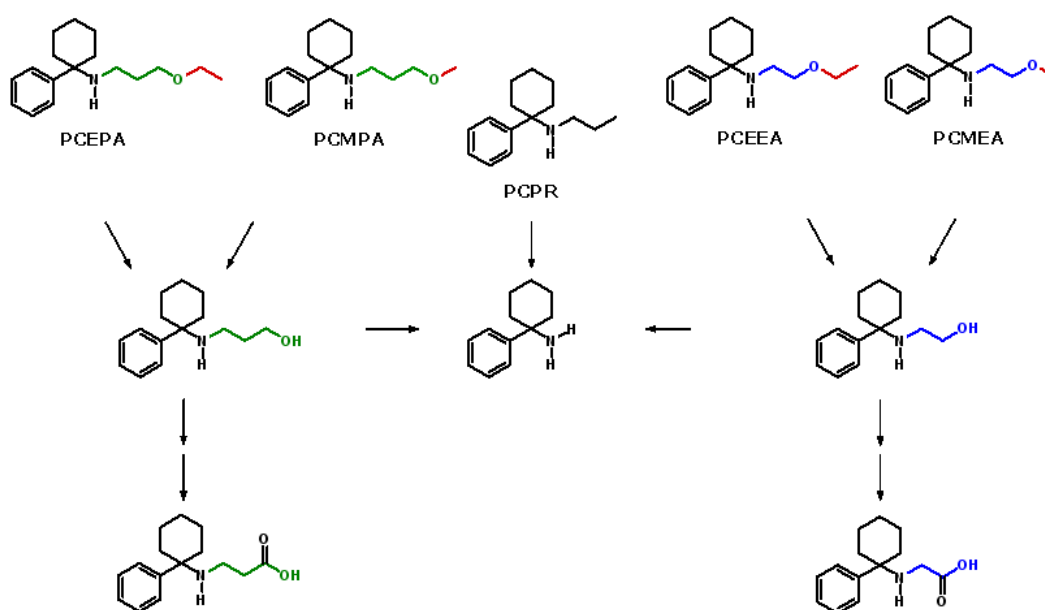


Fig. 1: Proposed scheme for the common metabolic pathway of the PCPs in rats.

3.2. Toxicological detection by GC-MS

The authors' STA procedure allowed the detection of an intake of a dose of the PCPs that corresponds to a common drug users' dose in urine. Fig. 2 shows typical reconstructed mass chromatograms with the given ions of an acetylated extract of a rat urine sample collected over 24 hours after application of 0.1 mg/kg BM of PCEEA and PCEPA, respectively, which corresponded to a common users' dose of about 7 mg. The identity of peaks in the mass chromatograms was confirmed by computerized comparison of the underlying mass spectrum with the reference spectra. After low dose application, all studied drugs were detectable by STA via their metabolites.

3.2. Initial screening studies

Among the nine CYPs tested, only CYP2B6, CYP2C19, CYP2D6 and CYP3A4 were markedly capable of catalyzing the O-dealkylation of PCEPA and PCMPA, respectively. For catalyzing the O-dealkylation of PCMEA CYP1A2, CYP2B6, CYP2C9, CYP2C19, and CYP2D6, could be detected. For catalyzing the O-dealkylation of PCEEA CYP1A2, CYP2B6, CYP2C9, CYP2C19, and CYP3A4 could be detected. In case of PCPR only CYP2B6 could be detected catalyzing the N-dealkylation, the ω -1 hydroxylation of the side chain, and hydroxylation of the cyclohexyl ring in position 4.

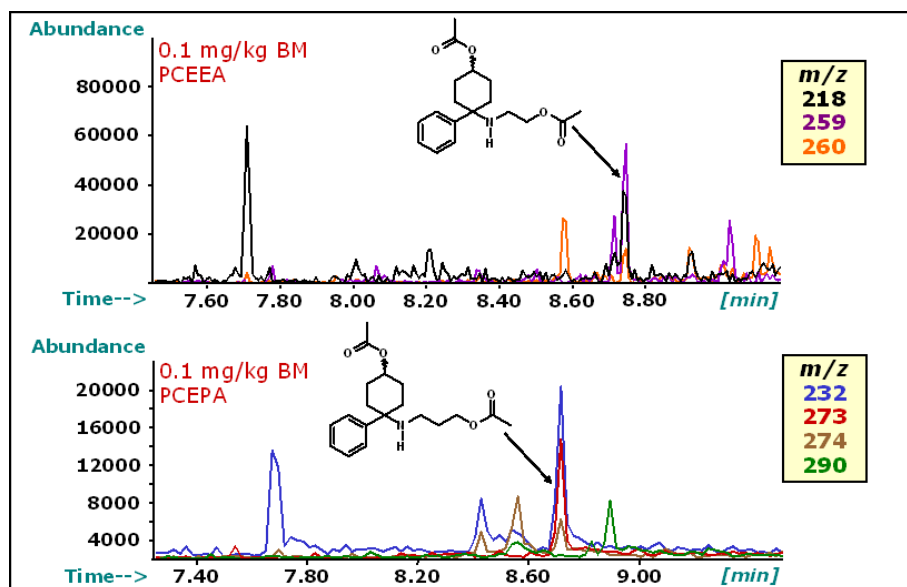


Fig. 2: Typical reconstructed mass chromatograms with the given ions of an acetylated extract of a rat urine sample collected over 24 hours after application of 0.1 mg/kg BM of PCEEA and PCEPA, respectively, which corresponded to a common drug users' dose of about 7 mg. The identity of peaks in the mass chromatograms was confirmed by computerized comparison of the underlying mass spectrum with the reference spectra.

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