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Studies on the Metabolism and the Detectability of Camfetamine in Rat Urine Using GC-MS and LC-(HR)-MS^n Techniques*

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1. Introduction

N-methyl-3-phenyl-norbornan-2-amine (camfetamine; CFA) is sold as a so-called "research chemical" via the internet and belongs as an amphetamine-type stimulant (ATS) to the group of new psychoactive substances (NPS). CFA is an analogue of *N*-ethyl-3-phenyl-norbornan-2-amine (fencamfamine; FCF), which was developed in the 1960's as an appetite suppressant and a stimulant for treating depressive day-time fatigue, or lack of concentration. Both compounds are camphor derivatives with an amphetamine backbone. FCF is scheduled in several countries, e.g. USA and Germany, but CFA is still uncontrolled. DeLucia et al. described FCF as central nervous stimulant and inhibitor of the dopamine reuptake and releaser of dopamine and noradrenalin [1]. Several other publications described its effects on body weight and addiction potential [2-6]. *N*-Deethyl FCF was the main metabolite described in man and para-hydroxy-aryl FCF in equine [7,8]. So far, no scientific information about the mechanism of action or the metabolism of CFA was published. CFA is described in internet forums (www.bluelight.com, www.eve-rave.ch, www.land-der-traeume.de), as a stimulant or a working aid providing increasing vigilance with typical amphetamine-like side effects. Therefore, CFA should be integrated into drug screening methods used in clinical and forensic toxicology. Kavanagh et al. published mass spectral data only of the synthesized standard [9], but metabolism data are needed for urine screening, as most central active drugs are excreted in a more or less completely metabolized form.

The aims of this study were to identify the phase I and II metabolites of camfetamine in rat urine and human liver microsome preparations by gas chromatography-mass spectrometry (GC-MS) and/or liquid chromatography-high resolution-linear ion trap mass spectrometry (LC-HR-MS^n) and to study the detectability of a common users' dose in urine within our standard urine screening approaches (SUSAs) using GC-MS [10-13] and LC-MS^n [14-16]. Finally, the human cytochrome-P450 (CYP) isoenzymes generally involved in the main metabolic steps should be identified.

2. Methods

The methods were already described by Welter et al. [17]. Urine samples were collected over 24 h from male Wistar rats shortly after administration of camfetamine for toxicologic diagnostic reasons. For the metabolism studies (20 mg/kg BW), urine samples were worked-up

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either by protein precipitation according to Wissenbach et al. [15], or by enzymatic conjugates cleavage and solid-phase extraction (HCX) for identification of phase I metabolites [10]. For the identification of phase II metabolites the samples were worked-up by C18 solid-phase extraction. The underivatized and/or acetylated extracts were then analyzed by GC-EI-MS (AT GC-MSD) or LC-high resolution (HR)-MS^n (TF Velos Orbitrap). For SUSA a low dose rat urine after administration of 3 mg/kg BW, which correspond to a described users' single dose of 30 mg [18] was worked-up by acid hydrolysis, liquid-liquid extraction and acetylation (GC-MS) or protein precipitation (LC-MS^n; TF LXQ). The conditions for the microsomal incubations were as follows: CFA (250 µM) was incubated with the CYP isoenzymes (75 pmol/mL, each) CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, or CYP3A5 for 30 min as well as HLM (50 mg protein/mL) as a positive control. After incubation, the solution was centrifuged and the supernatant analyzed using LC-HR-MS^n.

3. Results

Using GC-MS, it was possible to identify the following metabolites: nor CFA, two isomers of nor-hydroxy-alkyl CFA, two isomers of hydroxy-alkyl CFA, two isomers of hydroxy-aryl CFA, nor-hydroxy-aryl CFA, nor-hydroxy-methoxy CFA, and hydroxy-methoxy CFA. Most of those metabolites could be confirmed with the LC-HR-MS^n technique. Those which could not be confirmed, most probably due to insufficient separation or ionization, were one isomer of the nor-hydroxy-alkyl metabolites and one isomer of the hydroxy-alkyl metabolites. The mass spectral data and their interpretation are given in ref. [17]. The main metabolic pathways for CFA proposed according to the identified metabolites are shown in Fig. 1.

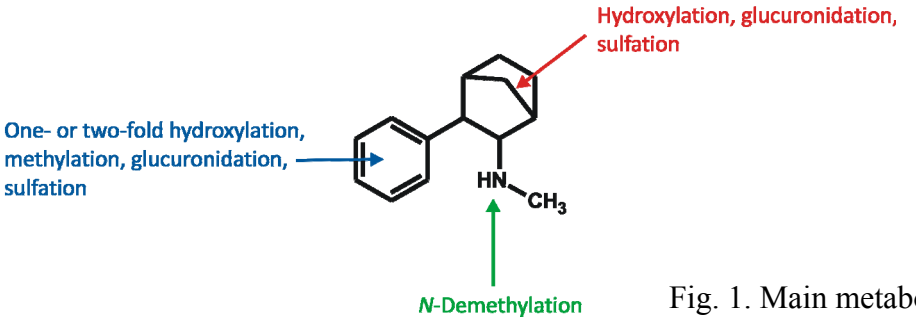

Fig. 1. Main metabolic pathways for CFA.

In detail, aromatic hydroxylation at two different, not specified, positions could be deduced followed by glucuronidation or sulfation. A second aromatic hydroxylation could be observed followed by methylation of one of the hydroxy groups and glucuronidation. Hydroxylation at two different not specified positions of the aliphatic ring system followed by glucuronidation of only one isomer could be seen. Another important step was the *N*-demethylation, and this metabolite underwent the same metabolic steps as CFA itself: aromatic hydroxylation at two different positions, glucuronidation and sulfation of one of the isomers; a second hydroxylation and methylation followed by glucuronidation, aliphatic hydroxylation at two different positions followed by glucuronidation and sulfation of one isomer.

Aromatic hydroxylation and *N*-demethylation were the predominant metabolic pathways for CFA in rat considering the relative GC-MS and LC-MS peak areas. Unfortunately, no reference standards were available for CFA; therefore quantification was not possible. Nevertheless, according to Delbeke et al., for FCF the described main metabolite in humans, was the nor metabolite, and in equine the hydroxy-aryl, the nor-hydroxy-aryl, and the corresponding glucuronides [7,8]. The HLM incubations showed the same results, as nor CFA, considering the relative peak areas, was the most abundant metabolite followed by hydroxy-aryl CFA.

By both SUSAs, CFA and several metabolites were detectable after low dose application. With the GC-MS SUSA CFA itself and hydroxy-aryl, nor-hydroxy-aryl, and nor CFA were detectable as most abundant metabolites. With the LC-MS^n SUSA, additionally two glucuronides were detectable as further targets, namely hydroxy-aryl CFA glucuronide and nor-hydroxy-aryl CFA glucuronide.

The initial CYP activity screening showed, that CYP2B6, CYP2C19, CYP2D6, and CYP3A4 catalyze the *N*-demethylation step, whereas CYP2C19 and CYP2D6 were found to be capable of catalyzing the aromatic hydroxylation and CYP1A2, CYP2B6, CYP2C19, and CYP3A4 the aliphatic hydroxylation.

4. Conclusion

Camfetamine was extensively metabolized and thus its metabolites were beside the parent compound the targets for urinalysis. Assuming similar metabolism in humans, both SUSAs should be suitable to prove an intake of camfetamine in human urine. Various CYP isoenzymes were involved in the different metabolic steps.

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