1,2-Diphenylethylamine Designer Drugs

Metabolism Studies and Toxicological Analysis Using Gas Chromatography-Mass Spectrometry and Liquid Chromatography-Mass Spectrometry Coupled to Low and High Resolution-Mass Spectrometry

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Sancta Simplicitas!

Mephistopheles in Faust - Johann Wolfgang von Goethe

Der Tragödie Erster Teil

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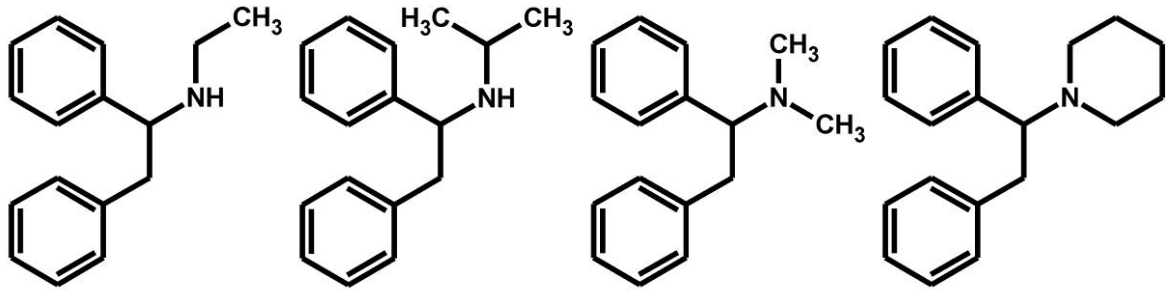
1. General Part

1.1 Introduction

1.1.1 Diphenylethylamines

The consumption of drugs has ever been part of human behavior. Plants and fungi with their large spectrum of active constituents have been either used to cure illness or abused to modulate body functions. In relation to that, one may think of hallucinogenic shamanic rituals, central nervous system (CNS) stimulation of caffeine (in *Coffea Arabica)* or ephedrine (in *Ephedra sinica)*, but also the variety of effects produced by opium (in *Papaver somniferum)*. With the ongoing scientific research new possibilities came up. The ingredients of plants and fungi were analyzed and tested for pharmacological effects. From that knowledge, classes of drugs were chemically synthesized in order to find new drugs for medical usage. A phenethylamine derivative with sympathomimetic effects (3,4-methylenedioxymethamphetamine, MDMA) was invented in 1912 by Merck Industries. It could have been used as an appetite suppressant but has never been marketed due to e.g. vascular side effects.[1] In spite of that, it is misused as an entactogenic CNS stimulating, controlled drug, known as Ecstasy.[2] So the stimulating and also cardiovascular effects of phenylethylamines have already been known for over a hundred years. Due to these pharmacological properties, diphenylethylamines were further investigated in the early 1940's in order to find new therapeutic drugs with fewer side effects. The chemical structures of such diphenylethylamines are depicted in Fig. 1. Tainter et al. studied 1,2-diphenylethylamine (DPEA) and several of its derivatives for CNS stimulation,[3] while Dodds et al. tested this compound class for its analgesic effects.[4] Approximately 20 years later, receptor binding and nuclear magnetic resonance (NMR) studies were conducted by Sasaki et al.[5] According to these findings, the (1R)-*N*,*N*-dimethyl-1,2-diphenylethanamine (lefetamine, Fig. 1c) was marketed as an opioid-like analgesic (Santenol, L-SPA) in Japan. Although first signs of withdrawal symptoms have been recorded,[6] it was marketed in the 1980's in Italy, but has later been scheduled due to dependency and withdrawal.[7-10] The core structure stayed interesting for the development of new designer drugs: In 2008, *N*-ethyl-1,2-diphenylethylamine (NEDPA, Fig. 1a) and *N-iso*-propyl-1,2-diphenylethylamine (NPDPA, Fig. 1b) were confiscated by the German police. In Sweden and Japan 1-(1,2-diphenylethyl)piperidine (diphenidine, Fig. 1d) has

been associated with drug induced intoxications. Due to that, several studies including synthesis, analysis, receptor binding and metabolism have been conducted for characterization of these compounds.[11-15] In many countries, such structurally similar drugs, often called “legal highs” or “research chemicals”, are not scheduled but may nevertheless lead to intoxications of drug abusers. In recent years more and more of such chemically derived compounds from various known drug classes have been recognized in order to circumvent the law.



a b c d

Fig. 1: NEDPA (a), NPDPA (b), lefetamine (c), diphenidine (d)

1.1.2 Pharmacology

Phenethylamines influence the signal transduction at (nor-)adrenergic, serotonergic, and dopaminergic receptors by inhibition of the transmitter reuptake. So, neurotransmitters stay in the synaptic cleft instead of being reabsorbed to presynapses, and can steadily bind to their receptors. This is the reason for the CNS stimulating effects and for tachyphylaxis, but maybe also for neurotoxicity.[2] According to the chemical structure, diphenylethylamines are also influencing the signal transduction. Additional to the CNS stimulation, effects such as bronchodilatation, hypertension, tachyphylaxis comparable to amphetamine have been described.[3,5]

Dodds et al. investigated the analgesic effects of diphenylethylamines according to the similarity to morphine and codeine (phenanthrene core)[16,17] Typical opioid effects were recorded: depression of righting reflex in mice, hyperexcitability, miosis, increased blood sugar levels, and nausea in cats. DPEA showed pain relieving properties in patients.

Pharmacologically, lefetamine was tested in the 1960’s in Japan, both the racemic compound and the enantiomers separately. Especially the L-isomer showed analgesic, antitussive, antipyretic, hypertensive, anticholinergic, vasoconstrictive, and local anesthetic effects. Furthermore, blood sugar was increased, barbiturate effects were

intensified and an inhibition of monoaminoxidase (MAO) was observed.[18] These results were further confirmed 20 years later by Italian working groups: Janiri et al. tested lefetamine after neocortical application to rats and reported opioid agonism and glutamate antagonism. Also the opioid and CNS stimulating effects in patients were published.[9,10] Afterwards, they tested lefetamine in order to reduce withdrawal symptoms with low effects compared to buprenorphine and clonidine.[8] De Montis et al. confirmed the opiate activity by the fact that naloxone pretreatment abolished the described effects. Further *in vitro* investigations showed that lefetamine displaced opioid receptor agonists from their binding site.[7] Diphenidine was shown to act as NMDA receptor blockers similar to ketamine, a therapeutically used narcotic also known as a drug of abuse.[13,15,19] Also DPEA, which was already known to antagonize glutamate receptors as described above, and new derivatives of diphenidine were tested substrates.

In summary, diphenylethylamines mainly showed CNS stimulating and opioid effects *in vitro* and *in vivo*.

1.1.3 Metabolism

After absorption, in most cases a drug needs to be metabolized to detoxify it, make it unlikely to be reabsorbed, clearable from blood system, and pharmacologically ineffective. Enzymes are responsible for these so-called biotransformations. A huge amount of them is located in the liver, but also in kidneys, gastrointestinal tract, skin, and bronchia. Hydrophilicity is often enhanced and so the substance can be excreted via urine, the most important way of excretion besides feces or exhaling.

Phase I metabolism represents the modification via oxidation, reduction, or hydrolysis. For example, hydroxy groups can be introduced into a molecule by an enzyme class called oxygenases. Monooxygenases, a subset of oxygenases, transfer NAD(P)H or FAD-dependent one oxygen atom into a molecule. This reaction is necessary in different metabolism steps: oxidation of a carbon-hydrogen bond to an alcohol, epoxidation of double bindings, or aromatic hydroxylations. Alcohols are reduced to corresponding aldehydes or ketones. So, phase I metabolism leads to functionalized and more hydrophilic compounds.

Monooxygenation and also reduction are often conducted by the hemoprotein group of cytochrome P450 (CYP). The name arose from the absorption band at 450 nm.[20] The CYPs are sorted by their amino acid sequence, divided into three subgroups, according

to their homology: An Arabic number at first titles the family, followed by a letter that defines the subfamily, and at last an Arabic number again for the member of subfamily. CYP3A4, the most important example for drug metabolism, is the fourth member of subfamily A in family three.[21,22] Currently, 18 CYP families, 43 subfamilies and 57 members of the subfamilies are known.[23] In humans, the greatest amount of CYPs is located in liver, but also in intestine, lung and brain. As they are part of membranes, liver preparations such as S9 or microsomes contain a comparable spectrum of the naturally occurring composition. As described above, commercially available cell systems can be used for *in vitro* studies.[24] To check for the involvement of a single enzyme in this compartment, the others can be blocked with known inhibitors,[25] and co-substrates of other enzyme reactions are omitted. But apart from those cell systems, CYP isozymes can be heterologously cDNA-expressed in bacteria or yeast for example.[26] This bears the opportunity of direct information if a substance is turned over by a certain enzyme, even in a low amount. As enzymes can be inhibited or induced by another drug or food, their activity can be influenced through genetic variations and interactions might occur. Poor and ultrarapid metabolizers gained interest in the efficacy and toxicity of pharmaceutical drugs such as codeine or tamoxifen.[27,28] CYPs often involved in xenobiotic metabolism should be tested, like CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4.[22] To avoid over-estimation of that involvement, a correction can be calculated as will be described in the kinetic chapter.

Phase II metabolism follows functionalization if necessary. Conjugation with large hydrophilic molecules such as glucuronic acid or sulfates leads to improved crossing urine or bile passage. Either water solubility is enhanced by this step or pH-dependent ionization prevents reabsorption.[22] Uridine diphosphate glucuronyltransferases catalyze the transfer of glucuronic acid to form glucuronides while sulfotransferases transform a sulfonate moiety to sulfate conjugates. These two enzyme groups represent the most important ones for the phase II metabolism of xenobiotics. Furthermore, catechol-*O*-methyltransferase (COMT) is a well-known enzyme from endogenous metabolism of neurotransmitters, leading to pharmacologically inactive compounds by methylation of hydroxy groups. This methylation prevents the binding to the respective receptors. According to the chemical structure, every compound with a similar catechol structure might be substrate of this enzyme as shown for e.g. 3,4-methylenedioxymethamphetamine.[29,30]

Investigating drug metabolism can be performed using *in vitro* or *in vivo* studies. *In vitro* assays can be conducted in two different ways: on the one hand, cell systems like S9 or human hepatocytes can be used to simulate general phase I or even phase II metabolism of a specific compound. Details on involved enzymes can be evaluated by using single human expressed isozymes for their involvement.[24,31-35] Even with these applicable systems, *in vivo* approaches are still necessary. They provide useful information not only on metabolizing steps, but also on absorption, distribution, and excretion.[22] *In vitro-in vivo* correlations can be conducted for confirmation of cell system results in animal studies.[36]

For lefetamine and its derivatives (Fig.1 a-c), neither *in vitro* nor *in vivo* metabolism studies have been published up to now. For diphenidine (Fig. 1d), small insights in metabolism were described by Wasaki et al. in consequence of intoxications.[12] Hydroxylations were the detected steps: mono-hydroxylation at the phenyl and at the piperidine moiety, combination of both steps and a bis-hydroxylation followed by dehydrogenation. All metabolites were detectable in urine and blood. Other phase I or phase II metabolites were not mentioned. In this study also quantitation was performed, which provided the following results: In blood and urine, both the parent compound was most abundant; mono-hydroxylation at the piperidine moiety was shown to be the most prominent metabolizing step. According to the lack of information, the elucidation by systematic metabolism studies for the diphenylethylamines was needed.

1.1.4 Kinetic Studies of Diphenylethylamines

The overall effectiveness of a certain enzyme to metabolize a drug depends on two aspects, the affinity to the enzyme and the enzyme capacity. The affinity of a drug to the enzyme is reported by the Michaelis-Menten-constant K_m. It is defined as the substrate concentration that leads to half maximum turnover velocity. With v_{max}, the capacity, the maximal velocity of a turnover activity is described. Within the linear range of time and protein concentration, the formation rate is maximal resulting in constant metabolite formation independent of the substrate concentration. The product formation is measured, and with reference standards, the concentration of formed metabolites can be calculated. Using Michaelis-Menten equation (eq. 1), the kinetic parameters and profile are defined as:

$$v = (v_{max} * [S]) / (K_m + [S]) \qquad (1)$$

The K_m and v_{max} values of single isozymes can be compared to each other, also after inhibition. To avoid overestimation and to account for their distribution in human liver, a relative activity factor approach should be conducted using human liver microsomes (HLM).[37]

1.1.5 Synthesis of Lefetamine

Lefetamine is part of schedule I of the Narcotics Law in Germany, the synthesis, habit and sale are prohibited. As it was not commercially available, it had to be synthesized in a modified version of Eschweiler-Clarke via bis-methylation of the primary amine (DPEA).[38] Using column chromatography for separation and purification, identity was checked and yield was calculated using liquid-chromatography (LC)-high resolution (HR)-mass spectrometry (MS) and NMR.[36] This substance was then used for the metabolism and kinetic studies *in vitro and in vivo*.

1.1.6 Detection, Structure Elucidation, and Quantification in Body Fluids

There are different ways to elucidate the chemical structure of compounds. MS can be used for structure elucidation over a wide mass to charge ratio. According to classic fragmentation rules, it can be done via the recorded spectra. This is especially true for HR-MS as it provides the possibility to measure the accurate (fragment) mass and propose the elemental composition of compounds or fragments. However, as fragmentation spectra may not provide detailed information of molecule, NMR could be conducted to confirm proposed structures by MS but also to provide additional information on exact positions.

According to the high sensitivity of MS, hyphenated techniques such as gas-chromatography (GC)-MS or LC-MS are well established methods in clinical and forensic laboratories. For detection and unambiguous identification, several reference libraries were available.[39-43] Using these, the analysis of samples can be done nearly automated as library search can be integrated in workflows.[44,45] Urine is easy to access and concentrated by a factor of about ten compared to blood, which makes it to the preferred matrix to use. Some analytes are metabolized to such an extent that it is difficult to detect them in their unchanged form in urine.[46-48] While urine provides an extended detection window for xenobiotics, blood, plasma, and serum samples are often used to access the presence and concentration, which might explain the

pharmacological effects. Plasma is cleared from parent compounds rather fast, either by metabolization or redistribution into fat tissues, metabolites might be excreted in urine over days up to weeks.[49-53] If only screened for the unchanged xenobiotic in the sample, false negative results might be produced.[42,54-57] In these cases, knowledge of metabolites is mandatory for toxicological analysis. Therefore, the metabolites detected in the studies for this dissertation, were included in the unique metabolite based reference libraries.[58-60]

2. Aims and Scopes

1,2-Diphenylethylamines have been synthesized and tested for their pharmacological activity soon after the effects of phenethylamines were known. Several years after marketing lefetamine (*N*,*N*-dimethyl-1,2-diphenylethylamine), the substance has become controlled. Lately, chemical derivatives of it were confiscated by the police in Germany, but no detailed information concerning these compounds was available. In Japan and Sweden, another derivative, namely diphenidine, was part of fatal intoxications, which already led to studies. As the abuse of designer drugs is relevant in clinical or forensic cases, parameters like metabolism, detectability and kinetics should be studied using common techniques in order to ensure their interpretation of toxicity. These aims and scopes were realized by:

- Studies on the metabolism and detectability of lefetamine-derived 1,2-diphenylethylamines using GC-MS, LC-MS^n, and LC-HR-MS/MS

- Synthesis of lefetamine, the pharmaceutical lead of diphenylethylamines, and confirmation of it by NMR

- Studies on the metabolism, confirmation of the proposed phase I metabolites using human liver preparations, and detectability of lefetamine using GC-MS, LC-MS^n, and LC-HR-MS/MS

- Investigation of toxicokinetic parameters regarding the initial metabolites of lefetamine and its derived derivatives

- Studies on the metabolism, confirmation of the proposed phase I metabolites detected in rat urine using human liver preparations, and detectability of diphenidine using GC-MS, LC-MS^n, and LC-HR-MS^n

3. Publication of the results

The results of the studies were published in the following papers:

3.1 Lefetamine-derived designer drugs *N*-ethyl-1,2-diphenylethylamine (NEDPA) and *N*-*iso*-propyl-1,2-diphenylethylamine (NPDPA): Metabolism and detectability in rat urine using GC-MS, LC-MS^n and LC-high resolution (HR)-MS/MS[61] (DOI 10.1002/dta.1621)

3.2 Lefetamine, a controlled drug and pharmaceutical lead of new designer drugs: Synthesis, metabolism, and detectability in urine and human liver preparations using GC-MS, LC-MS^n, and LC-high resolution-MS/MS^{36} (DOI 10.1007/s00216-014-8414-3)

3.3 Toxicokinetics of lefetamine and derived diphenylethylamine designer drugs – Contribution of human cytochrome P450 isozymes to their main phase I metabolic steps[62] (DOI 10.1016/j.toxlet.2015.08.012)

3.4 Diphenidine, a new psychoactive substance: Metabolic fate elucidated with rat urine and human liver preparations and detectability in urine using GC-MS, LC-MS^n, and LC-HR-MS^n[63] (DOI 10.1002/dta.1946)

4. Conclusions

NEDPA and NPDPA, the two lefetamine-derived designer drugs, were shown to be extensively metabolized in rats. After application of a high dosage of the drugs, *N*-dealkylation, mono- and bis-hydroxylation of the benzyl-ring, combination of those steps and also the combined *N*-dealkylation and hydroxylation of the phenyl moiety were for both drugs the proposed phase I metabolism steps. As conjugated compounds, glucuronides and sulfates of the hydroxy and hydroxy-methoxy metabolites could be detected. No case reports of abuse have been known, so the dosage for the studies of detectability using standard urine screening approaches (SUSA) of the author's lab was scaled-up from the medically used lefetamine (Santenol).[58,60,64] In the rat urine, collected for 24 hours, *N*-dealkyl, *N*-dealkyl-hydroxy, and hydroxy metabolites were detectable by GC-MS SUSA. Using LC-MS^n SUSA, NEDPA intake was revealed by the glucuronides of mono- and bis-hydroxy, N-deethyl-hydroxy and hydroxy-methoxy metabolite, for NPDPA only by hydroxy glucuronide. Some interesting aspects came up while studying these derivatives: first, GC-MS fragmentation provided an alpha-cleavage, so hydroxylation at phenyl- or at benzyl-moiety was definable. In contrast, in LC-MS (electrospray ionization) the loss of the nitrogen part was the initial fragmentation step. Thus, the daughter spectra of corresponding hydroxy metabolites (at phenyl and benzyl moiety) obtained at different retention times, were identical. All GC-MS proposed metabolites could be confirmed by LC-HR-MS/MS, and additionally glucuronides and sulfates which are not detectable by GC-MS. The second important point is that there are common metabolites after consumption of NEDPA, NPDPA, lefetamine, or diphenidine, namely those after *N*-dealkylation. But the detectability studies presented at least one unique metabolite, so the differentiation after intake of one of those applied drugs should be possible.[61]

After the urgent studies on the confiscated drugs NEDPA and NPDPA, still no information of the metabolism or detectability of the pharmaceutical lead lefetamine has been known. It is scheduled in the narcotics act because of its known effects, but not commercially available as reference standard. With permission, it was synthesized via bis-methylation starting with DPEA, which is legally available. After isolation and purification, synthesized lefetamine was qualitatively and quantitatively characterized by LC-HR-MS and NMR, so it could be used for *in vitro* and *in vivo* metabolism studies and for determination of enzyme kinetic profiles. After application of a 25 mg/kg BW dose of

lefetamine to rats, urine was collected over 24 hours and analyzed by GC-MS and LC-HR-MS/MS. The following metabolizing steps could be detected: *N*-oxidation, mono- and bis-*N*-dealkylation, mono- and bis-hydroxylation at various positions of the aryl moieties, combinations of those steps, glucuronidation, sulfation, and methylation of one of the hydroxy-groups by COMT. In summary, the metabolic pathways already investigated for NEDPA and NPDPA could also be found for lefetamine. The proposed *N*-oxide, *N*-demethyl, *N*,*N*-bis-demethyl, *N*-demethyl-hydroxy, and hydroxy metabolites of rat urine studies were confirmed in HLM, the dealkylated metabolites also in human liver cytosol (HLC). For the study of detectability, the applied dosage of lefetamine was scaled-up as already described for the derivatives. Besides *N*,*N*-bis-demethyl, *N*,*N*-bis-demethyl-hydroxy, *N*-demethyl-hydroxy, *N*-demethyl-bis-hydroxy and its corresponding glucuronide, lefetamine itself was detectable in rat urine. So in case of co-consumption of lefetamine as controlled substance and one of its "legal high" derivatives, differentiation should be possible. As mentioned before, all metabolite spectra have been implemented into existing metabolite-based GC-MS and LC-MS libraries to enhance the detectability and to allow differentiation of applied drugs.[36,58-60]

As drugs are often co-consumed with medication or other drugs, interactions (e.g. for biotransformations of these) might occur. Therefore, the involvement of the ten most important CYPs in the initial *N*-dealkylation was determined.[62] The three drugs NEDPA, NPDPA, and lefetamine were incubated with baculovirus-infected insect cell microsomes of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and CYP3A5. CYP1A2, CYP2B6, CYP2C19, CYP2D6, and CYP3A4 were capable of forming the initial metabolite of lefetamine and NEDPA, for NPDPA the formation of its metabolite by CYP2D6 was too low to achieve. After the development of a quantitative method, kinetic profiles of metabolite formation were investigated and found to follow classic Michaelis-Menten kinetics with K_m between 2 and 331 µM and v_{max} between 4 and 36 pmol/min/pmol CYP450 enzyme. With application of the relative activity factor (RAF) approach to avoid overestimation of those parameters, the contribution of a single isozyme in the whole cell compartment of HLM was calculated.[34] Net clearances were about 72% by CYP 2B6, 17% by CYP3A4 and less than 10% for CYP1A2, CYYP2C19, and CYP2D6 for lefetamine. For NEDPA/NPDPA the percentage of net clearance was calculated as 27/18 %, 30/24 %, 23/28 %, and 17/30 % for CYP1A2, CYP2B6, CYP2C19, and CYP3A4, respectively and for NEDPA additionally by 2D6 by 4%. Chemical inhibition was in line with *in vivo*

contributions. As there are at least four CYP isozymes involved in one initial step of metabolism, there should be a low clinical relevance of interactions. However, individual polymorphism in CYPs, other drugs and food contents co-consumed, and the endogenous transporters may increase the risk of clinical relevant interactions. This could only be estimated by further studies or evaluated from case reports.

The last studied diphenylethylamine was also confiscated by the German police. After identity was confirmed to be diphenidine by HR-MS and NMR and purity was quantified by qNMR, studies on metabolism in rat and human liver preparations were conducted. Mono- and bis-hydroxylation at aryl and also at piperidine moiety, followed by dehydrogenation of the hydroxy-piperidine to an oxo-metabolite, were the one-step metabolites detected. *N*-dealkylation and combinations of it with hydroxylation were the additional reactions for phase I metabolism. Glucuronides and hydroxy-methoxy compounds turned over by COMT represented the conjugated phase II metabolites. Fragmentation was comparable to already studied 1,2-diphenylethylamines: An alpha-cleavage from nitrogen in GC-MS allowed the differentiation of hydroxylation at the aryl or at the piperidine ring. The metabolites after *N*-dealkylation were the same as those of lefetamine, NEDPA and NPDPA, so comparable with the underlying spectra of MS libraries. All the GC-MS detected metabolites could be confirmed using LC-HR-MS^n. The initial, oxygenated metabolites in rat urine were also built in HLM and HLC. To fulfill metabolic elucidation and predict interaction, a CYP assay was passed. CYP1A2, CYP2B6, CYP2C19, and CYP3A4 formed hydroxy-aryl, hydroxy-piperidine, and bis-hydroxy-piperidine metabolites, while CYP2D6 was only involved in the turnover to mono-hydroxy metabolites. With this knowledge of metabolism, urinalysis for detectability was conducted afterwards with doses scaled up from case reports of Japan. Several metabolites were detected using the different SUSA: by GC-MS: the oxo (so the dehydrogenated hydroxy-piperidine part), hydroxy-piperidine, oxo-hydroxy-phenyl, two hydroxy-phenyl-hydroxy-piperidine, and *N*-dealkyl-hydroxy-methoxy metabolites; and by LC-MS^n: oxo, hydroxy-aryl, and bis-hydroxy-piperidine for phase I and for phase II hydroxy-methoxy and the glucuronides of hydroxy-methoxy and mono-hydroxy-aryl metabolites.

5. Summary

In the presented studies, metabolism and detectability were investigated of designer drugs belonging to the class of 1,2-diphenylethylamines. NEDPA, NPDPA, and diphenidine were confiscated drugs, while lefetamine had to be synthesized first. The identity and purity of the drugs were at first confirmed by HR-MS and NMR studies. Common phase I metabolic pathways of NEDPA, NPDPA, lefetamine and diphenidine were *N*-dealkylation, mono- and bis-hydroxylation at ring moieties, combination of both steps. Additionally, lefetamine and diphenidine were *N*-oxidized. Glucuronidation and catechol methylation for phase II metabolism were shown for all substances, for NEDPA, NPDPA, and lefetamine, but sulfates only for NEDPA, NPDPA, and lefetamine. In detectability studies, each drug intake resulted in common but also specific metabolites allowing detection and differentiation of the four tested compounds. The initial phase I metabolites detected in rat urine could mostly be confirmed in HLM. CYP1A2, CYP2B6, CYP2C9, CYP2C19, CP2D6, and CYP3A4 were the enzymes mainly involved the initial metabolic steps of 1,2-diphenylethylamines. For NEDPA, NPDPA, and lefetamine also enzymatic turnover was elucidated, which showed that they all followed classic Michaelis-Menten kinetics.

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7. Abbreviations

cDNA	copy deoxyribonucleic acid
CNS	central nervous system
COMT	catechol-*O*-methyltransferase
CYP	cytochrome P450
DPEA	1,2-Diphenyethylamine
e.g.	exempli gratia, for example
GC	gas chromatography
HLC	human liver cytosol
HLM	human liver microsomes
HR	high resolution
LC	liquid chromatography
L-SPA	Lefetamine
MALDI	matrix assisted laser desorption ionization
MAO	monoamineoxidase
MDMA	3,4-methylenedioxymethamphetamine
MS	mass spectrometry
NEDPA	*N*-ethyl-1,2-diphenylethylamine
NMDA	*N*-methyl-D-aspartate
NMR	nuclear magnetic resonance spectroscopy
NPDPA	*N*-iso-propyl-1,2-diphenylethylamine
SUSA	Standard urine screening approach(es)
UN	United Nations
WHO	World Health Organization

8. Zusammenfassung

In den hier vorgestellten Studien wurden der Metabolismus und die Nachweisbarkeit von vier Designerdrogen aus der Klasse der 1,2-Diphenylethylamine untersucht. Dafür wurden konfisziertes NEDPA, NPDPA und Diphenidin eingesetzt, während Lefetamin zuerst synthetisiert werden musste. Die Identität und Reinheit der verwendeten Stoffe wurden unter anderem durch HR-MS und NMR belegt. Gemeinsame Metabolisierungsschritte von NEDPA, NPDPA, Lefetamin und Diphenidin waren die *N*-Dealkylierung, Ein- und Zweifachhydroxylierung an den Ringstrukturen sowie die Kombination aus beiden Schritten. Zusätzlich wurden für Lefetamin und Diphenidin *N*-Oxide nachgewiesen. Alle Substanzen zeigten ausgeprägten Phase II Metabolismus (Glukuronidierung und Catechol-Methylierung). Für NEDPA, NPDPA und Lefetamin konnten zusätzlich Sulfatkonjugate detektiert werden. Die Einnahme der vier Drogen konnte im Urin mittels gemeinsamer Metabolite nachgewiesen werden, spezifische erlaubten eine Unterscheidung. *In vitro* Experimente mit humanen Lebermikrosomen konnten die in vivo Resultate aus Rattenurin größtenteils bestätigen. CYP1A2, CYP2B6, CYP2C9, CYP2C19, CP2D6 und CYP3A4 waren hauptsächlich an der Umsetzung der 1,2-Diphenylethylamine beteiligt. Diese setzten NEDPA, NPDPA und Lefetamin nach klassischer Michaelis-Menten-Kinetik um.