Summary of the PhD Thesis [1] as 'Thank You' for the GTFCh Travel Fund for Presenting at the 2011 SOFT-TIAFT Meeting in San Francisco (CA)

In vivo and *in vitro* metabolism studies of glaucine, a new herbal high by GC-MS, LC-MS, LC-HR-MS, and NMR techniques

Golo M. J. Meyer and Hans H. Maurer

Abteilung Experimentelle und Klinische Toxikologie, Universität des Saarlandes, D-66421 Homburg-Saar, Germany

Abstract: The isoquinoline alkaloid Glaucine was described as an ingredient of legal highs and gained the interest of clinical and forensic toxicologist. So far, only few data on its pharmacokinetic properties was investigated. The aims of the present studies were to elucidate the metabolic fate of glaucine *in vivo* (rat) by GC-MS and LC-HR-MS^n techniques, to confirm the human main phase I metabolites *in vitro*, to identify the involved CYP isoenzyme using heterologically expressed single CYPs, to determine the Michaelis-Menten constants K_m, and V_{max} in human liver microsomes, and finally, to investigate the detectability by the authors standard urine screening approaches. In rats, glaucine was mainly *O*- and *N*-dealkylated followed by conjugation to glucuronides or sulfates. The following CYP isoforms were mainly involved in these reactions: CYP1A2, CYP2C19, CYP2D6, CYP3A4, and CYP3A5. The kinetic profiles of all metabolite formations followed classic Michaelis-Menten behavior and the K_m values were between 25-140 µM and the V_{max} values between 0.10 - 1.92 pmol/min/pmol. Toxicological detection should be focused on the demethyl metabolites and the corresponding glucuronides and/or sulfates.

1. Introduction

The isoquinoline alkaloid Glaucine (structure given in Fig. 1) is one of several new "herbal highs", which are used as drugs of abuse by the drug scene [2]. Herbal highs are single compounds or mixtures of different compounds with more or less strong psychoactive effects and often with non-declared ingredients [3]. Commonly, these compounds are not scheduled and neither the seizin nor the consumption is regulated by the narcotic law. Therefore, these compounds were used as legal highs. In the last decade, several new "legal highs" were available especially in the internet or in so called head shops. Mostly, the identity of such preparations was not confirmed and their pharmacological and toxic effects as well as their pharmacokinetic behavior are unknown. Therefore, studies on the metabolism and the detectability of the compounds and their metabolites in body samples of such "herbal highs" play an important role in clinical and forensic toxicology.

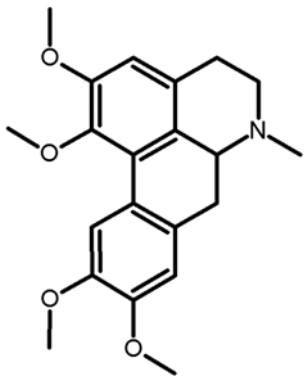

Fig. 1. Glaucine structure.

As summarized by Meyer [1], glaucine ((*S*)-5,6,6a,7-tetrahydro-1,2,9,10-tetramethoxy-6-methyl-4H-dibenzo[de,g] quinoline is an isoquinoline alkaloid with aporphine structure and main ingredient of *Glaucium flavum* (yellow hornpoppy, *Papaveraceae*). The plant species is native in Western Europe, North America, and Asia and the latex, typical for *Papaveraceae*, contains the main alkaloids in varying concentration (up to 3 %) [4]. Other alkaloids of the

species are chelerythrine, magnoflorine, protopine and other minor ones [5]. Glaucine was described to have anti-inflammatory and antitussive properties, inhibits the phosphodiesterase-4 (PDE4), acts as bronchodilator, calcium channel blocker, and weak dopamine D_1 and D_2 receptor antagonist [6;7]. The antitussive properties of glaucine in humans were investigated in comparison to codeine or dextromethorphan [8] and it showed a comparable decrement in cough besides less adverse effects and no signs of opiate withdrawal after longer intake [9]. Case reports of recreational use [10; 11] or side effects of therapeutic use [2] are described and show the following symptoms: feeling of tiredness, hallucinations, vomiting, dizziness, and decreased blood pressure. Next to its therapeutic use, Glaucine is part of misused party pills or legal highs [2; 10].

2. Material and Methods

After administration of glaucine to rats, urine samples were collected over 24h, worked-up by different protocols, and analyzed by GC-MS and LC-HR-MS [12]. Due to the lack of differentiations of the corresponding demethylated isomeric metabolites by MS technique, four of five possible structures were synthesized and characterized by NMR [13]. Subsequently, the main phase I metabolites were confirmed in humans by incubation of glaucine with pooled human liver microsomes (pHLM) and the involved isoenzyme were determined by incubation of Glaucine in heterologically expressed single CYPs. The kinetic profiles were recorded for pHLM and all mainly involved CYPs by the metabolite formation approach. The results were compared with data of simple peak area ratios of analytes and internal standards. For testing the detectability, low doses of glaucine were administered to rat and urines were analyzed by both authors standard urine screening approaches (GC-MS and LC-MS^n) [12].

3. Results and Discussion

After extensive interpretations of the fragmentation of glaucine and its corresponding metabolites, 26 phase I and 21 phase II metabolites could be identified [12]. Glaucine was mainly *O*- and *N*-dealkylated in rats and further conjugated to glucuronides or sulfates. Further metabolic steps were *N*-oxidation, and hydroxylations, as well as combinations of them. The synthesized demethylated metabolites, the structures of which were confirmed by NMR [13], allowed their identification in urine extracts by comparing their chromatographic retention times and mass spectra. The demethylated metabolites were also identified to be the main targets in both toxicological urine screening approaches [14; 15]. An intake of glaucine could be monitored in rat urine after administration of 2 mg/kg body mass (BM), corresponding to a 40 mg human single cough medication dose scaled by dose-by-factor approach according to ref. [16]. Using the GC-MS approach, the acetylated mono- and bis-demethylated metabolites could be detected whereas in the LC-MS^n screening approach the demethylated metabolites and their glucuronides were the analytical targets [12].

The further in vitro studies with HLM confirmed the formation of the mono-demethylated metabolites in humans [13]. The following CYP isoforms were mainly involved in these reactions: 1A2, 2C19, 2D6, 3A4, and 3A5. The K_m values were between 25-140 µM and the V_{max} values between 0.10 - 1.92 pmol/min/pmol. All kinetic profiles could be modeled using the Michaelis-Menten equation. For all enzyme kinetics (CYPs, HLM), the metabolite formation approach using calibration with the corresponding synthesized reference standards was applied and compared to the results to those obtained by the other isomers and arbitrary units (peal area analyte vs. internal standard). The bias of the quantification approach for assessing the contribution of CYPs to hepatic clearance and therefore to the hepatic net clearance in

vivo was negligible [13]. Further inhibition experiments in HLM with specific and selective inhibitors confirmed the results of the contribution of the involved CYPs. Using the Michaelis-Menten equation and determined pharmacokinetic constants, concentration-dependent involvement of CYP isoforms was calculated for different glaucine concentrations (0.1-100 µM) according to Meyer et al. [17]. In a case report of recreational glaucine use [10], plasma levels of 0.7 mg/L (2 µM) were documented. Over the tested concentration range, only minor variations in the enzyme contributions were observed. The overall contribution of all CYP-catalyzed metabolic steps was as follow: CYP3A4 was about 79 %, of CYP1A2 18 %, of CYP2D6 2 %, and of CYP2C19 <1 % determined for a glaucine concentration of 5 µM.

4. Conclusion

As concluded by Meyer [1], glaucine was extensively metabolized in rats and in human liver preparations. The determination method for recording the kinetics had no substantial impact on the estimation of *in vivo* hepatic clearance of glaucine. Based on numbers of metabolic reactions and several involved CYP isoforms in the hepatic clearance the risk of clinical relevant reactions with single inhibitors should be low. Such risks, e.g. drug-drug or drug-food interactions, as well as genetic variations were important for assessment for clinical relevant interactions [17-20]. Especially for drugs of abuse, these interactions might be more relevant, because many drug addicted were polytoxicomania and consumed several drugs, leading in a higher risk of such interactions.

5. References

[1] Meyer GMJ. Herbal Drugs of Abuse - *Glaucium flavum and Sceletium tortuosum*: Metabolism and toxicological detectability of their alkaloids glaucine, mesembrine and mesembrenone studied in rat urine and human liver preparations using GC-MS, LC-MS, LC-HR-MS^n, and NMR. Dissertation, Saarland University, Saarbruecken 2014.

[2] Geppert B, Wachowiak R, Zaba C. Glaucine as a non-declared active component of "Legal Highs". Problems Forensic Sci 2010;84:401-8.

[3] Gibbons S. 'Legal highs'--novel and emerging psychoactive drugs: a chemical overview for the toxicologist. Clin Toxicol (Phila) 2012;50:15-24.

[4] Petitto V, Serafini M, Gallo FR, Multari G, Nicoletti M. Alkaloids from Glaucium flavum from Sardinia. Nat Prod Res 2010;24:1033-5.

[5] Daskalova E, Iskrenova E, Kiryakov H G, Evstatieva L. Minor Alkaloids of Glaucium flavum. Phytochem 1987;27:953-5.

[6] Cortijo J, Villagrasa V, Pons R, Berto L, Marti-Cabrera M, Martinez-Losa M, et al. Bronchodilator and anti-inflammatory activities of glaucine: In vitro studies in human airway smooth muscle and polymorphonuclear leukocytes. Br J Pharmacol 1999;127:1641-51.

[7] Asencio M, Hurtado-Guzman C, Lopez JJ, Cassels BK, Protais P, Chagraoui A. Structure-affinity relationships of halogenated predicentrine and glaucine derivatives at D1 and D2 dopaminergic receptors: halogenation and D1 receptor selectivity. Bioorg Med Chem 2005;13:3699-704.

[8] Dicpinigaitis PV, Morice AH, Birring SS, McGarvey L, Smith JA, Canning BJ, Page CP. Antitussive drugs--past, present, and future. Pharmacol Rev 2014;66:468-512.

[9] Zhang A, Zhang Y, Branfman AR, Baldessarini RJ, Neumeyer JL. Advances in development of dopaminergic aporphinoids. J Med Chem 2007;50:171-81.

[10] Dargan PI, Button J, Hawkins L, Archer JR, Ovaska H, Lidder S, et al. Detection of the pharmaceutical agent glaucine as a recreational drug. Eur J Clin Pharmacol 2008;64:553-4.

[11] Lidder S, Dargan P, Sexton M, Button J, Ramsey J, Holt D, Wood D. Cardiovascular toxicity associated with recreational use of diphenylprolinol (diphenyl-2-pyrrolidinemethanol [D2PM]). J Med Toxicol 2008;4:167-9.

[12] Meyer GMJ, Meyer MR, Wissenbach DK, Maurer HH. Studies on the metabolism and toxicological detection of glaucine, an isoquinoline alkaloid from Glaucium flavum (Papaveraceae), in rat urine using GC-MS, LC-MSn and LC-high-resolution MSn. J Mass Spectrom 2013;48:24-41.

[13] Meyer GMJ, Meyer MR, Wink CSD, Zapp J, Maurer HH. Studies on the in vivo contribution of human cytochrome P450s to the hepatic metabolism of glaucine, a new drug of abuse. Biochem Pharmacol 2013;86:1497-506.
[14] Wissenbach DK, Meyer MR, Remane D, Weber AA, Maurer HH. Development of the first metabolite-based LC-MSn urine drug screening procedure - exemplified for antidepressants. Anal Bioanal Chem 2011;400:79-88.
[15] Maurer HH, Pfleger K, Weber AA. Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and their Metabolites, 4th ed. Weinheim (Germany): Wiley-VCH, 2011:
[16] Sharma V, McNeill JH. To scale or not to scale: the principles of dose extrapolation. Br J Pharmacol 2009;157:907-21.
[17] Meyer MR, Peters FT, Maurer HH. The role of human hepatic cytochrome P450 isozymes in the metabolism of racemic 3,4-methylenedioxy-methamphetamine and its enantiomers. Drug Metab Dispos 2008;36:2345-54.