Designer drug TMA-2: studies on the metabolism including CYP isoform dependency and on the toxicological detection

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AIMS: 2,4,5-trimethoxyamphetamine (TMA-2) is a designer drug which has appeared on the illicit drug market in Europe and the USA. In the meantime, TMA-2 has been scheduled in the German Act of Controlled Substances and in many other countries. The aim of our study was to identify its metabolites, the cytochrome P450 (CYP) isoenzymes involved in the main metabolic step, and to examine the toxicological detection in rat urine by the authors' GC-MS STA procedure.

METHODS: TMA-2 (20 mg/kg body mass for identification of metabolites, 0.5 mg/kg body mass for toxicological detection) was given to male Wistar rats by gastric intubation and urine was collected over a 24 hour period. The metabolites were isolated either after enzymatic cleavage of conjugates or directly by liquid-liquid extraction (LLE, pH 8-9). The metabolites (either underivatized or acetylated) were separated and identified by GC-MS in the EI and PICI mode. For studies on the toxicological detection acid hydrolysis, LLE and acetylation was performed (details in Ewald/Fritschi/Bork/Maurer, J. Mass Spectrom. 41, 487, 2006). Studies on the identification of the CYP isoenzymes involved in the O-demethylation were performed by 30 min incubations of TMA-2 with nine single cDNA expressed enzymes (started by adding enzyme microsomes, terminated with perchloric acid) and analysis by LC-MS without further workup (details in Staack/Paul/Springer/Kraemer/Maurer, Biochem. Pharmacol. 67, 235, 2004).

RESULTS: Eleven metabolites of TMA-2 could be identified by interpretation of the respective mass spectra detected in the urine extract after enzymatic hydrolysis and acetylation. All metabolites could also be detected to a minor extent in the native urine extract. O-demethylation, the main metabolic step, was catalyzed exclusively by CYP2D6. In the STA, the O-demethyl-TMA-2 isomers were the target analytes for monitoring abuse.

CONCLUSIONS: According to the identified metabolites, the following metabolic pathways could be postulated: TMA-2 undergoes single or double O-demethylation, oxidative deamination, oxidative deamination and further reduction to the corresponding alcohol, or single O-demethylation combined with oxidative deamination. The metabolites were found to be partly excreted as glucuronide and/or sulfate conjugates. As the main metabolic step was catalyzed exclusively by CYP2D6, variations in hepatic drug elimination may possibly occur due to CYP2D6 poor or ultra rapid metabolism or interactions with CYP2D6 inhibitors. Considering similar metabolism and kinetics of rats and humans an intake of a common dose of TMA-2 should be detectable by STA.

KEYWORDS: *TMA-2, Designer drug, Metabolism, CYP*

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