# Identification of human monoamine oxidases A/B and cytochrome P450 2D6 as major enzymes involved in the main metabolic steps of phenethylamine-derived designer drugs (2c-series)

DENIS S. THEOBALD and HANS H. MAURER,

Department of Experimental and Clinical Toxicology, University of Saarland, D-66421 Homburg (Saar), Germany

**AIMS:** In recent years, several compounds of the so-called 2C-series have entered the illicit drug market as designer drugs. In former studies, the qualitative metabolism of important 2C's using a rat model was studied and the metabolic pathways were deduced. Main phase I metabolic steps were deamination, O-demethylation and side chain hydroxylation. The aim of this study was to determine the monoamine oxidase (MAO) and cytochrome P450 (CYP) isoenzymes involved in these main metabolic steps and to measure the Michaelis Menten kinetics of the deamination reactions.

**METHODS:** The isoenzyme-dependency of the metabolism of the following substances was studied: 2C-B, 2C-D, 2C-E, 2C-I, 2C-T-2, and 2C-T-7. For this purpose, initial screening studies with 50 pmol/ml CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, 0.2 mg/ml MAO-A, or 0.2 mg/ml MAO-B were performed. Incubation mixtures (final volume 50 µl) for initial screening studies consisted of 100 mM phosphate buffer (pH 7.4), 250 µM substrate at 37°C, and exclusive for CYP incubations, 5mM isocitrate, 1.2 mM NADP+, 0.5 U/ml isocitrate dehydrogenase, and 200 U/ml superoxide dismutase. Reactions were started by addition of the ice-cold microsomes and terminated with 5 µl of 60% (w/w) aqueous $HClO_4$.

The kinetic studies were carried out at initial rate conditions, a prerequisite for Michaelis-Menten kinetics. Optimal MAO-A and MAO-B protein contents and incubation times for each single substrate had been derived from preliminary studies. For the kinetic studies, incubation mixtures (final volume 50 µl) consisted of 100 mM phosphate buffer (pH 7.4), variable substrate concentrations and different MAO-A/MAO-B protein concentrations (depending on the substrate) at 37°C.
The samples were extracted with 50 µl of cyclohexane containing 0.01 mM of the internal standard 2,5-dimethoxybenzaldehyde for detection of the respective aldehydes resulting from deamination, and with a mixture of dichloromethane-isopropanol-ethyl acetate (1:1:3; v/v/v) at pH 8-9 to extract other metabolites. The latter organic phase was evaporated and derivatized by acetylation. 2 µl of the cyclohexane extract and 2 µl of the derivatized extract were injected into the GC-MS operated in SIM mode.
Apparent Km and vmax values were estimated by nonlinear regression according to the Michaelis-Menten equation: $V=Vmax \bullet [S]/(Km + [S])$. The kinetic parameters were estimated using GraphPad Prism 3.02 software (GraphPad software Inc., San Diego, CA).

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**RESULTS:** Among the 11 MAO and CYP enzymes tested for possible formation of aldehydes from the respective parent compounds, MAO-A and MAO-B were the main enzymes capable of catalyzing these reactions in all tested drugs. For some compounds, CYP2D6 also catalyzed this reaction but only to a very minor extent. CYP2D6 catalyzed also the O-demethylation and hydroxylation. Apparent MAO-A and B Km and vmax values of the deamination step are presented for each substrate.

**CONCLUSIONS:** The results of this study showed that MAO-A, MAO-B and CYP2D6 are the major phase I enzymes involved in the metabolism of the studied 2C compounds. The 2C's might therefore be susceptible for CYP2D6 polymorphisms and for drug-drug interactions with MAO and CYP2D6 inhibitors.

**KEYWORDS:** *2C-series, MAO, CYP, Isoenzymes, Designer drug, Metabolism, GC-MS*

**Corresponding author:** hans.maurer@uniklinikum-saarland.de