Major review

Inhibitory metabolite complex formation of methylenedioxymethamphetamine with rat and human cytochrome P450. Particular involvement of CYP 2D

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Abstract

Methylenedioxymethamphetamine (MDMA or ecstasy) is a common recreational drug used at rave parties. Unfortunately, MDMA may have neurological effects and in some cases causes hepatotoxicity. MDMA binds to cytochrome P450 in rat and human hepatic microsomal preparations. Upon metabolic transformation of either the methylenedioxy or the methylamino function, it forms an inhibitory P450-metabolite complex. This inhibitory complex is formed predominantly with the P450 2D isozymes. This complex formation may account for the clinical toxicity observed upon ingestion of MDMA, particularly with other compounds normally metabolized by P450 2D6. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Methylenedioxymethamphetamine (MDMA or ecstasy) is a common recreational drug used at rave parties. Reports estimate that millions of young people use MDMA at least occasionally (Green et al., 1995; Grob et al., 1995; Schwartz and Miller, 1997). The toxicity of MDMA is well documented in animals and humans, and in some cases causes death (Berger et al., 1992; Coore, 1996; Aguirre et al., 1997). In addition to malignant hyperthermia and neurological effects, hepatotoxic effects of MDMA have also been reported (deMan et al., 1993; Khakoo et al., 1995; Fidler et al., 1996; Ellis et al., 1996; Hellinger et al., 1997). Liver

function parameters spontaneously normalize upon withdrawal of the drug. The underlying mechanism of hepatitis has as yet to be clearly demonstrated by Khakoo et al. (1995).

Amphetamine derivatives are extensively metabolized into analogues of neurologically active amines such as dopamine by cytochromes P450 in rat and human liver (see Cho and Kumagai (1994) for a review). MDMA (Fig. 1) is oxidized at its methylenedioxy group to form catechol and the amino function can be demethylated into the corresponding primary amine. Such metabolites have been identified in the urine of rats given MDMA (Lim and Foltz, 1988; Ensslin et al., 1996). Amphetamine metabolites are involved in the observed toxicological effects (Cho and Kumagai, 1994; Tomkins et al., 1997; Carvalho et al., 1997). CYP 2B and 2D P450 isozymes are involved in amphetamine metabolism (Cho and Kumagai, 1994; Kumagai et al., 1994), as shown directly by metabolic studies (Cho and Kumagai 1994; Kumagai et al., 1994; Tucker et al., 1994; Lin et al., 1997) and by inhibitory effects

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(Tomkins et al., 1997; Wu et al., 1997). P450 2B and 2D are present in both liver and brain, and so reactive metabolites such as catechol are formed directly near the neuronal target. Both amphetamine and methylenedioxy compounds are precursors of stable inhibitory complexes with P450 iron which absorb at 455 nm (Franklin, 1977; Lindeke and Cho, 1982; Cho and Kumagai, 1994; Ortiz de Montellano and Correia, 1995). The amine function can be oxidized into a nitroso metabolite which readily reacts with Fe(II), and methylenedioxy can be metabolized into carbene, thus forming stable Fe(II) or Fe(III) complexes. These P450-metabolite complexes are responsible for the inhibition of enzymatic activities observed in vivo and in vitro in animals treated with tertiary amino compounds or methylenedioxy derivatives (Delaforge et al., 1985; Bensoussan et al., 1995).

Cytochrome P450 2D6 is a polymorphic member of the cytochrome P450 superfamily and is absent in about 10% of Caucasians (Guengerich, 1995). We now provide direct evidence of the formation of a stable MDMA inhibitory complex with rat and human cytochrome P450, the 2D isozymes being predominantly affected. This formation of a stable inhibitory complex may result in perturbed liver metabolism and thus in hepatotoxicity, as in the case of macrolide antibiotics (Mansuy and Delaforge, 1993). This also suggests potent risks upon concomitant ingestion with drugs mainly metabolized by CYP2D.

2. Materials

Methylenedioxymethamphetamine, NADPH, NADP, glucose 6 phosphate (Glc6P), glucose 6 phosphate dehydrogenase (Glc6PDH), 3-methylcholanthrene, phenobarbitone, were from Sigma. Dexamethasone, clofibrate were from Janssen Chemicals. Other chemicals were of the highest purity available.

3. Methods

Male Sprague–Dawley (SD) rats (Iffa Credo, St Germain l'Arbresle, France) and Dark-Agouti rats (Janvier, France) were treated as described previously

MDMA

Fig. 1. Chemical formula of methylenedioxymethamphetamine or ecstasy.

(Bensoussan et al., 1995), and microsomes were stored at −80°C until use.

Yeast-expressed human P450s were produced in the presence of either yeast P450-reductase or human P450-reductase (Peyronneau et al., 1992; Pompon et al., 1995). The corresponding microsomes were prepared as described previously (Peyronneau et al., 1992). Human samples were kindly provided by P. Beaune (UA 390, INSERM, Paris)

Protein Lowry et al. (1951), P450, Omura and Sato (1964), and formaldehyde formation Bensoussan et al. (1995) were determined as already described.

Spectral interaction was monitored using a double beam Uvikon 941 spectrophotometer with 1 nmole P450/ml in a 1cm pathway cuvette (0.2 or 0.5 nmol P450/ml in the case of yeast-expressed human P450). The baseline was recorded between 520 and 350 nm using the differential mode by direct subtraction of the reference cuvette absorption from the sample cuvette absorption. MDMA solubilized in methanol was added to the sample cuvette (0.1–100 μM). The same volume of methanol was added to the reference cuvette. Difference amplitude spectra (394–417 nm) were recorded after curve smoothing and were plotted as a function of the concentration, in the double inverse mode (1/OD f 1/concentration), for the determination of the spectral dissociation constant (K_s) (Schenkman et al., 1981). Formation of 455 nm absorbing cytochrome P450 metabolite complex was measured by differential spectroscopy with microsomes containing 1 μM cytochrome P450 (0.15 μM in the case of yeast-expressed human P450), 0.1 mM substrate and 0.5 mM NADPH. Difference spectra were recorded from 1 ml cuvettes (1 cm pathlength) between 400 and 520 nm every 2 min and the maximum absorbance of the 455 nm absorbing cytochrome P450 metabolite complex was determined (Franklin, 1977; Lindeke and Cho, 1982).

N- and O-dealkylase activities were measured by formaldehyde formation according to a standard technique, Bensoussan et al. (1995), using 0.1 mM substrate, 1 μM P450 and an NADPH-generating system (1 mM NADP, 10 mM Pc6P, 1IU G6PDH) for 10 min at 37°C.

4. Results

4.1. Spectral interactions

Addition of MDMA to hepatic microsomes from untreated rats led to the formation of type I spectral interactions (λ_{max} 394 nm, λ_{min} 417 nm) (Fig. 2a, Table 1). This spectral interaction was also observed for MDMA, but less markedly, with microsomes from SD rats treated with phenobarbital, dexamethasone, 3-methylcholanthrene, clofibrate or isoniazid. This
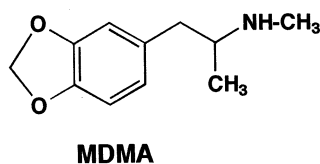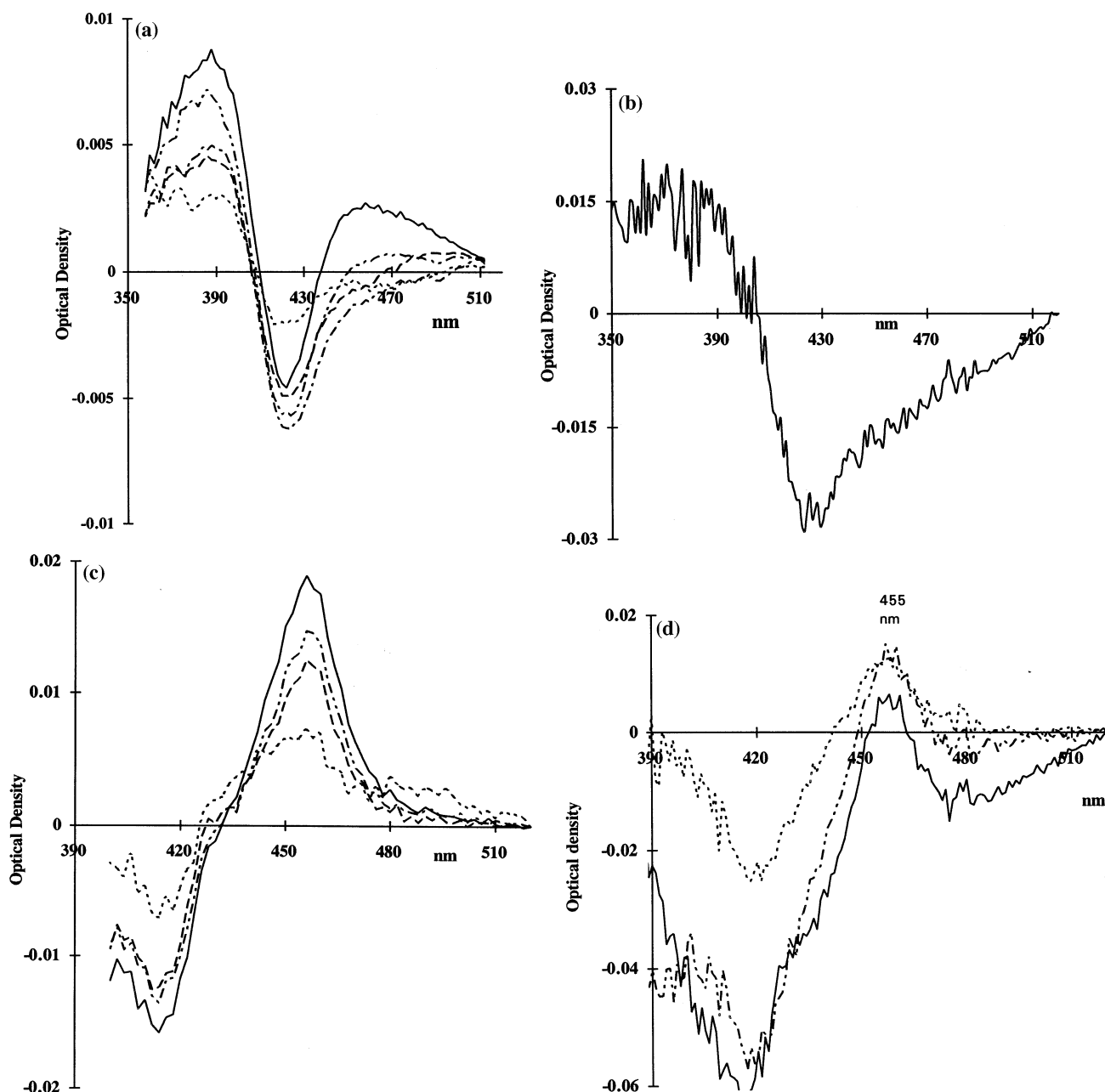
Fig. 2. Spectral interaction of methylenedioxymethamphetamine with microsomes from untreated rat liver or yeast-expressed human P450 2D6. (a) Difference spectra of 1 μM P450 from hepatic microsomes from untreated–treated rat in 0.1 M phosphate buffer. MDMA (1 to 100 μM) in ethanol was added to the sample cuvette. The spectrum was recorded immediately. 1 μM...., 5 μM·---, 10 μM $- \cdot - \cdot -$ 20 μM $- \cdot \cdot - \cdot \cdot$, 100 μM —— MDMA. (b) Difference spectra of 0.5 μM P450 from yeast-expressed human P450 2D6 in the presence of 90 μM MDMA.(c) Difference spectra obtained after further addition of 0.5 mM NADPH to a rat microsomal preparation and 100 μM MDMA to the sample cuvette (Fig. 2a). Spectra were recorded every 2 min for 20 min at 37°C (2....., 6----, 12 $- \cdot \cdot - \cdot \cdot$, 20 min ——). (d) Addition of NADPH to yeast-expressed CYP 2D6 (Fig. 2b). Spectra were recorded 2....., 6 $- \cdot \cdot - \cdot \cdot$, 20 min —— after addition of NADPH at 28°C.

strongly suggests that MDMA interacts preferentially with a P450 active site mainly present in untreated animals. The dissociation constant of MDMA with rat microsomes was measured at around 4 μM. Type I spectra were markedly inhibited in the presence of quinine or quinidine, with IC 50 of 2.10^{-6} and 10^{-5} M, respectively. Microsomal preparations of male Dark-Agouti rats were able to produce weak type I

spectra even at 100 μM MDMA (Table 1). This rat strain is known to possess very low amounts of P450 2D (Gonzalez et al., 1987).

The type I spectral interaction with MDMA was also observed using hepatic human microsomes (Table 1), with an associated K_s value of around 20 μM. Using human P450 isozymes expressed in yeast, spectral interaction was only observed with P450 2D6 (Fig. 2b).

4.2. Inhibitory metabolite—P40 complex formation

Incubation of untreated rat microsomes with MDMA, in the presence of NADPH at 37°C, led to the formation of a complex absorbing at 455 nm, levels of which increased with time (Fig. 2c). This complex was stable in the presence of reducing agents such as NADPH or dithionite. Upon addition of potassium ferricyanide, the absorption spectrum of the complex centred around 435 nm. The 435 nm absorbing complex was also formed upon incubation of microsomes with MDMA and cumene hydroperoxide. These properties were in accordance with the formation of a carbene–P450 complex arising from the oxidative metabolism of the methylenedioxy function (Lindeke and Cho, 1982, Delaforge et al., 1985). Measurements of the remaining amounts of P450 after MDMA incubation in the presence of NADPH indicated a loss of around 20% in comparison with the blank incubation performed in the absence of NADPH. The 455 nm absorbing complex formation was abolished in the presence of 10^{-5} M quinine or quinidine, indicating the possible involvement of P450 2D.

The comparison of inhibitory P450–metabolite complex formation by hepatic microsomal preparations from rats treated with various inducers is reported in Table 1. All the microsomal preparations were able to metabolize MDMA, but at a slower rate (25–60% of the untreated rats). P450 losses were less than when using untreated rat microsomes. Dark-Agouti rat microsomal preparations were able to form about one-third of the amount of the 455 nm inhibitory complex measured in Sprague–Dawley rats. This suggests that in addition to 2D isozymes, other P450 forms

were still able to form small amounts of this inhibitory complex.

Using hepatic human microsomes, less inhibitory complex was formed than with rat microsomes. The 455 nm absorbing complex was also observed when using yeast-expressed human CYP2D6 (Fig. 2d). As already observed with macrolide antibiotics, rat microsomes were more potent in vitro in forming the 455 nm absorbing complex, as compared to human liver microsomes or to human P450 expressed in yeast (personal observations).

N-demethylation or methylenedioxy cleavage of MDMA were also confirmed in rat and human microsomal preparations by formaldehyde measurements indicating oxidative demethylation of either the N-methyl or loss of the methylenedioxy function.

5. Conclusion

The formation of P450 metabolite complexes upon oxidation of drugs containing tertiary amine or methylenedioxy functions involves oxidation leading to inhibition of P450 catalytic activities. MDMA has the particularity of having on the same molecule both the tertiary amine and the methylenedioxy functions. It yielded significant amounts of 455 nm absorbing complexes with human and rat liver microsomes, arising mainly from the oxidation of the methylenedioxy function. This complex formation was abolished by quinine and quinidine, and was lower in Dark-Agouti rat microsomes, indicating the involvement of P450 2D. Using yeast-expressed human cytochrome P450, only P450 2D6 was able to form this inhibitory complex. On

Table 1
Spectral interactions of MDMA with hepatic microsomal preparations from human and from rat treated with various inducing agents

Microsomal preparation	Binding type[a]	ΔOD_{max} 390–420 nm[b]	Ks μM[c]	455–490 nm[d]	450 loss (%)[e]	Formaldehyde formation[f]
Untreated SD rat	I	0.014	4	0.019	19	1.65
Phenobarbital-treated SD rat	I	0.006	n.m.	0.008	10	0.2
Dexamethasone-treated SD rat	I	0.004	n.m.	0.012	14	0.95
3-Methylcholanthrene-treated SD rat	I	0.005	n.m.	0.007	9	0.45
Clofibrate-treated SD rat	I	0.009	3	0.005	10	0.90
Isoniazid-treated SD rat	I	0.011	5	0.010	11	1.10
Dark-Agouti untreated rat	I	0.008	n.m.	0.006	5	0.55
Human	I	0.015	20	0.005	10	1.0

[a] Type I binding refers to a spectral interaction of MDMA with the P450 active site and modification of the proportions of low- and high-spin iron absorbing, respectively at 417 and 394 nm Schenkman et al. (1981).

[b] Maximum absorption difference spectrum measured after successive addition of 1–100 μM MDMA in methanol to 1 nmol P450/ml.

[c] Dissociation constant (Ks) measured from a double reciprocal plot $1/\Delta OD_{390-420\ nm}$ f $1/(MDMA)$.

[d] 455 nm complex formation measured after 20 min at 37°C following addition of NADPH to a microsomal preparation (1 μM P450) containing 100 μM MDMA.

[e] Loss of P450 content measured after NADPH and MDMA incubations. Each determination is corrected for spontaneous loss of P450 observed in the presence of NADPH alone.

[f] Formaldehyde formation measured using the NASH technique Bensoussan et al. (1995).n.m.: not measurable.

coincubation of MDMA with testosterone, ethoxyre-sorufin or dextrometorphan, no significant inhibition was observed for 6β testosterone or resorufin formation catalyzed by P450 3A or 1A, respectively, but the dextrometorphan metabolism catalyzed by P450 2D was decreased by about 50% at 100 μM MDMA. This complex formation may be related to the hepatotoxic effects of MDMA, as already demonstrated for drugs having either a methylenedioxy (Franklin, 1977; Delaforge et al., 1985; Ortiz de Montellano and Correia, 1995) or a secondary or tertiary amino function (Franklin, 1977; Lindeke and Cho, 1982; Delaforge et al., 1985; Mansuy and Delaforge, 1993; Cho and Kumagai, 1994; Ortiz de Montellano and Correia, 1995). Such compounds led directly to lower metabolism of the drug in question or to the associated drug being metabolized by the same P450 isozyme.

This inhibitory complex formation can have dramatic consequences for MDMA disposition in 2D6-deficient people since this isozyme is mainly involved in MDMA metabolism. Both low amounts of CYP 2D and subsequent blockage of this isozyme led to very low metabolism of MDMA, higher plasma concentrations, and longer-lasting effects. This is particularly crucial in mothers with low amounts of 2D, Wadelius et al. (1997) and exacerbates the neurological or hormonal effects of MDMA (Grob et al., 1995; Aguirre et al., 1997).

Acknowledgements

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