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Inhibition of human cytochrome P450 2D6 (CYP2D6) by methadone

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1 In microsomes prepared from three human livers, methadone competitively inhibited the *O*-demethylation of dextromethorphan, a marker substrate for CYP2D6. The apparent K_i value of methadone ranged from 2.5 to 5 μM.

2 Two hundred and fifty-two (252) white Caucasians, including 210 unrelated healthy volunteers and 42 opiate abusers undergoing treatment with methadone were phenotyped using dextromethorphan as the marker drug. Although the frequency of poor metabolizers was similar in both groups, the extensive metabolizers among the opiate abusers tended to have higher *O*-demethylation metabolic ratios and to excrete less of the dose as dextromethorphan metabolites than control extensive metabolizer subjects. These data suggest inhibition of CYP2D6 by methadone *in vivo* as well.

3 Because methadone is widely used in the treatment of opiate abuse, inhibition of CYP2D6 activity in these patients might contribute to exaggerated response or unexpected toxicity from drugs that are substrates of this enzyme.

Keywords methadone CYP2D6 dextromethorphan oxidation phenotype human liver microsomes

Introduction

Genetically variable activity of cytochrome P450 2D6 (abbreviated CYP2D6, Nebert *et al.*, 1991) results in polymorphic oxidation of debrisoquine, sparteine, dextromethorphan and many other drugs (Eichelbaum & Gross, 1990; Lennard, 1990). The CYP2D6 enzyme is absent in 5 to 10% of the Caucasian population of Europe and North America as a result of *CYP2D6* gene deletion or mutations (Gonzalez & Meyer, 1991; Meyer *et al.*, 1990). Although CYP2D6 activity is not inducible (Eichelbaum & Gross, 1990), an increasing number of drugs and chemicals have been shown to be inhibitors of this enzyme (Brøsen & Gram, 1989). Among them, quinidine is a well characterized inhibitor which is very potent *in vitro* and *in vivo* (Otton *et al.*, 1984; Nielsen *et al.*, 1990). Several studies have demonstrated that a single oral dose of quinidine sulphate (50 to 250 mg) is sufficient to block the activity of CYP2D6 and to temporarily convert an extensive metabolizer (EM) into a poor metabolizer (PM) (Broly *et al.*, 1991; Inaba *et al.*, 1986; Leeman *et al.*, 1986; Nielsen *et al.*, 1990). Thus, while the activity of CYP2D6 is under genetic control, it is significantly influenced by environmental factors as well.

To identify inhibitors of CYP2D6, the effect of the test compound on the rate of metabolite formation of a prototype substrate (such as debrisoquine, sparteine, or dextromethorphan) by human liver microsomes is examined (Boobis *et al.*, 1983; Otton *et al.*, 1983, 1984). To date, about 200 drugs and chemicals have been submitted to this screening procedure (Fonne-Pfister & Meyer, 1988; Fonne-Pfister *et al.*, 1987; Inaba *et al.*, 1985).

During the course of *in vitro* screening of drugs of abuse for an interaction with CYP2D6, we observed that methadone potently inhibited dextromethorphan *O*-demethylation. Methadone had earlier been found to have no effect on CYP2D6 activity in human liver microsomes (Henthorn *et al.*, 1989), although Mikus *et al.* (1991) recently reported that it potently inhibits codeine *O*-demethylation by rat liver microsomes. In the present paper, we describe our *in vitro* findings and data suggesting that methadone in doses used clinically inhibits CYP2D6 activity *in vivo*.

Methods

Chemicals

Dextromethorphan hydrobromide, D-glucose-6-phosphate monosodium salt, NADP sodium salt, and

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glucose-6-phosphate dehydrogenase were purchased from Sigma Chemical Co. (St Louis, MO, USA). Dextrorphan, 3-methoxymorphinan, 3-hydroxymorphinan and levallorphan tartrate (internal standard) were kindly provided by Hoffman-La Roche Inc., Nutley, N.J., USA. Racemic methadone hydrochloride was supplied by the Pharmacy of the Addiction Research Foundation of Ontario, Canada. All other chemicals were of analytical reagent grade.

Human liver samples and preparation of liver microsomes

The characteristics of the donors of the three human livers (K21, K23 and K27) used in this study were described previously (Campbell *et al.*, 1987; Tyndale *et al.*, 1989). Microsomes were prepared and stored according to established techniques (Tyndale *et al.*, 1989). Microsomal protein concentration was determined using the BCA protein assay kit (Pierce Chemical Co., Rockford, IL, USA) and the bovine serum albumin standard solution provided.

Incubation conditions

The incubation conditions were essentially those of Otton *et al.* (1983). Dextromethorphan (final concentration 0.5 μM, 1.0 μM or 5.0 μM) was incubated with an NADPH generating system (0.1 μmol NADP, 0.2 units glucose-6-phosphate dehydrogenase, 1 μmol glucose-6-phosphate, and 0.5 μmol $MgCl_2$) in 0.2 M phosphate buffer (pH 7.4), and with 75 μg of microsomal protein. At each substrate concentration, methadone was added to final concentrations of 5 μM, 10 μM or 20 μM. The volume of the incubation mixture was 250 μl. Incubations were performed at 37° C in a shaking water bath for 30 min. Preliminary studies had demonstrated that the production of dextrorphan from 2 μM dextromethorphan was linear from 25 to 500 μg microsomal protein ml^{-1} incubation mixture and throughout a 30 min incubation. The reaction was stopped by the addition of 20 μl of 70% (w/v) perchloric acid and cooling the samples on ice. After centrifugation at 2500 rev min^{-1} for 10 min, 50 μl of the supernatant was assayed for dextrorphan by h.p.l.c. Dextrorphan concentration in the samples were stable for at least 6 days at room temperature.

Human subjects

Two-hundred and ten (210) unrelated healthy subjects (94 men and 116 women, aged 17 to 51 years) and 42 unrelated abusers of oral opiates (24 men and 18 women, aged 22 to 59 years) who were currently undergoing treatment with methadone were recruited for this study. All subjects reported Caucasian ancestry and were living in the metropolitan area of Toronto. The abusers were in-patients at the Clinical Research and Treatment Institute of the Addiction Research Foundation and were taking 20 to 50 mg methadone daily. They were phenotyped 2 to 6 days after the initiation of the methadone treatment. In addition to methadone, seven patients took 10 mg diazepam daily, two took 0.1 mg clonidine four times per day, and three patients took ranitidine as adjunctive treatment for opiate withdrawal. All subjects signed consent forms, and the research protocol was approved by the Ethics Committee of the Addiction Research Foundation. Each subject took a single oral dose (30 mg) of dextromethorphan hydrobromide at bedtime and collected 0–8 h urine the following morning. An aliquot of urine was stored at −20° C until h.p.l.c. analysis.

H.p.l.c. assay

Dextromethorphan and three metabolites 3-hydroxymorphinan, 3-methoxymorphinan, and dextrorphan were measured as described by Chen *et al.* (1990) except that the excitation and emission wavelengths were set at 195 nm and 280 nm, respectively, and a phenyl column (5 μm, 15 × 0.46 cm, Chromatography Sciences Company, Montreal, Canada) was used for the separation. Dextrorphan formed *in vitro* was measured using the same assay. No interferring peaks were formed from methadone during the incubation.

Data analysis

The apparent inhibitor constant (K_i) of methadone was calculated by the equation of Dixon & Webb (1964). Dixon and Cornish-Bowden plots were used to indicate the type of inhibition. In the population study, the *O*-demethylation metabolic ratios (ODMR) in the control subjects and patients treated with methadone were calculated as % of dose (dextrorphan plus 3-hydroxymorphinan)/(dextromethorphan plus 3-methoxymorphinan), and plotted as a frequency distribution histogram with the logarithm of ODMR as abscissa. The phenotype frequencies in the methadone group was compared with the control frequencies by Chi-square tests. The percentage of the dose eliminated as dextromethorphan and its metabolites was recorded as mean ± s.d. Statistical analyses of the differences between phenotype groups and subject groups were carried out using the Mann Whitney U Test. All analyses were performed using PHARM/PCS (Tallarida & Murray, 1984) or JMP (version 2, SAS Institute Inc., 1989).

Results

In vitro *inhibition of CYP2D6 by methadone*

As shown in Figure 1, methadone competitively inhibited dextromethorphan *O*-demethylation by human liver microsomes. Microsomes from the three human livers (K21, K23 and K27) were tested twice on different occasions. The mean K_i values of methadone for each of the three livers were 4 μM, 2.8 μM, and 3.5 μM, respectively, with a range from 2.5 to 5 μM.

In vivo *inhibition of CYP2D6 by methadone*

The *O*-demethylation of dextromethorphan was clearly polymorphic in the control group of subjects (Figure 2), as has been reported by others (Faccini *et al.*, 1990; Larrey *et al.*, 1987; Schmid *et al.*, 1985). The frequency of PMs was about 7% (15 of 210 subjects). This was not significantly different from the PM frequency among

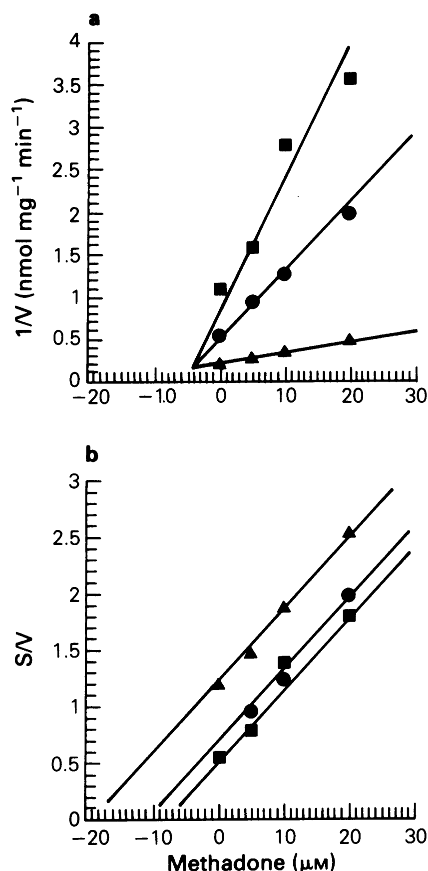

Figure 1 Dixon (a) and Cornish-Bowden (b) plots showing competitive inhibition of dextromethorphan *O*-demethylation by methadone in microsomes from one human liver (K21). Lines were drawn by linear regression analysis; the correlation coefficients were > 0.97. ■ DM 0.5 μM, ● DM 1.0 μM, ▲ DM 5.0 μM.

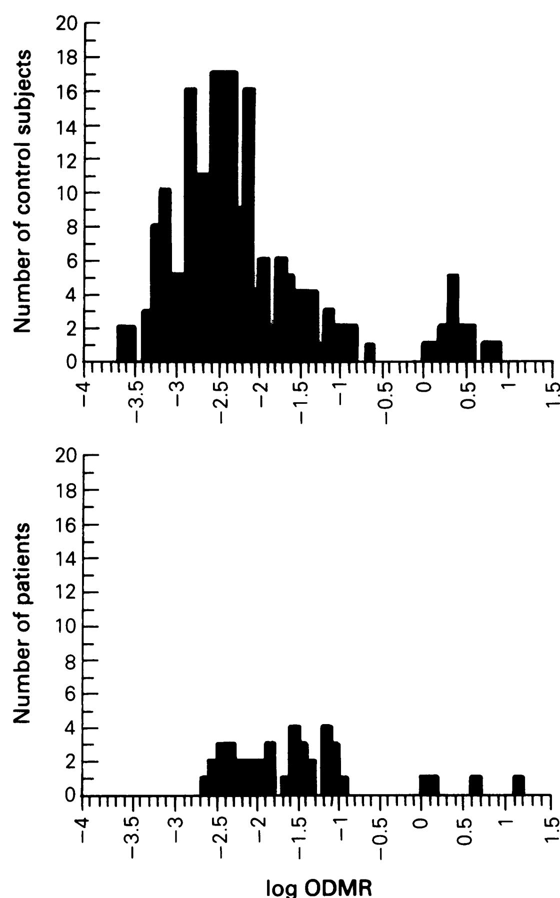

Figure 2 Frequency distribution of the logarithm of dextromethorphan *O*-demethylation ratios (ODMR) in 210 control subjects (a) and in 42 methadone-treated patients (b). The ODMRs were calculated as the amounts of (dextrorphan plus 3-hydroxymorphinan)/(dextromethorphan plus 3-methoxymorphinan) in overnight urine.

opiate abusers taking methadone for withdrawal (9.5%, four of 42 subjects, $P > 0.05$ by Chi-square test). However, the mean ODMR value among opiate abusers who comprised the lower mode of EMs (i.e. those subjects having log ODMR values < -0.3) was significantly higher than that observed for the 195 control EMs (log ODMR -1.783 ± 0.517 in EM patients *vs* log ODMR -2.341 ± 0.607 in EM control subjects, $P < 0.001$). The urinary output (0–8 h) of dextromethorphan and metabolites in the PM patients was not statistically different from that in the PM controls (Table 1). However, the EM patients tended to excrete less of the dose as dextrorphan and 3-hydroxymorphinan and more as 3-methoxymorphinan and dextromethorphan than the EM control subjects. As a result, the total recovery as percentage of the dose in the EM patients was intermediate between that seen in EMs and PMs of the control population.

Discussion

Although Henthorn *et al.* (1989) reported that methadone is not an inhibitor of hepatic CYP2D6 activity, we found that this drug potently inhibits dextromethorphan *O*-demethylation in microsomes from three human livers. The reason for this discrepancy is unknown, but it may be related to the different CYP2D6 substrates

Table 1 Comparison of the urinary output (0–8 h) of dextromethorphan and metabolites in 210 control subjects to that in 42 opiate abusers who took methadone for withdrawal. Data are expressed as the mean percentage of the dose ± s.d.

	Control subjects		*Patients*		P *value*	
	(*EMs* n = 195)	(*PMs* n = 15)	(*EMs* n = 38)	(*PMs* n = 4)	*EMs(C)* vs *EMs(P)*	*PMs(C)* vs *PMs(P)*
Dextromethorphan	0.22 ± 0.45	2.15 ± 0.94	0.32 ± 0.55	2.04 ± 1.27	< 0.001	0.881
Dextrorphan	21.18 ± 11.50	0.58 ± 0.32	15.50 ± 10.69	0.35 ± 0.37	< 0.005	0.229
3-Hydroxymorphinan	7.26 ± 7.78	0.24 ± 0.14	3.99 ± 5.83	0.37 ± 0.38	< 0.001	0.177
3-Methoxymorphinan	0.02 ± 0.03	0.15 ± 0.08	0.04 ± 0.04	0.25 ± 0.21	< 0.001	0.689
Total recovery	28.68 ± 18.05	3.13 ± 1.24	19.86 ± 14.86	3.03 ± 1.07	< 0.005	0.960

used (desipramine and dextromethorphan). In that same report, codeine had no effect on desipramine 2-hydroxylation, although codeine is firmly established as a CYP2D6 substrate (Dayer *et al.*, 1988; Desmeulles *et al.*, 1989; Mortimer *et al.*, 1990; Sindrup *et al.*, 1991; Yue *et al.*, 1987, 1989). Codeine is also a CYP2D6 inhibitor with a K_i value of 230 μM determined using dextromethorphan (Dayer *et al.*, 1989) and 90 μM using sparteine (Otton *et al.*, unpublished observation) as the substrate. In addition, two clinical reports (Kosten *et al.*, 1990; Maany *et al.*, 1989) have demonstrated that co-administration of methadone significantly increased plasma concentrations of desipramine, apparently because of inhibition of desipramine 2-hydroxylation.

In addition to the *in vitro* competition study, we also compared dextromethorphan metabolic ratios in opiate abusers who were receiving methadone for withdrawal with those in control subjects. It is unlikely that the shift towards lower CYP2D6 activity (as reflected by higher dextromethorphan ODMRs) amongst the EM patients resulted from concurrent medication. Only seven of the 38 EM patients were administered drugs other than methadone, and these drugs were either not CYP2D6 inhibitors e.g. diazepam (Inaba *et al.*, 1985) and ranitidine (Spina & Koike, 1986) or not potent inhibitors e.g. clonidine (Inaba *et al.*, 1985). None of the members of the opiate group was willing to repeat the ODMR determination a few weeks after completion of their methadone treatment.

Methadone itself is extensively *N*-demethylated by hepatic microsomal enzymes (Beckett *et al.*, 1968; Pohland *et al.*, 1971). Despite its widespread use, there are few clinical reports describing interactions between methadone and other drugs. In addition to its use in treatment of opiate abuse (Jaffe, 1986; Martin, 1977), methadone itself has been abused (Bell *et al.*, 1990; Kolar *et al.*, 1990). Since the half-life of methadone in humans is about 23 h (Inturrisi *et al.*, 1987), it will accumulate after multiple doses. The peak concentrations of methadone in the plasma in methadone maintenance patients were reported (Inturrisi & Verebely, 1972) to be about 860 $\mu g\ l^{-1}$ or 2.8 μM (MW 309.5), a concentration which falls in the range of the K_i values observed here. Therefore, although our data indicate that inhibition of CYP2D6 activity by methadone *in vivo* is incomplete, it still might contribute to clinically important drug interactions. Furthermore, unexpected toxicity might occur if methadone users concurrently use other psychoactive drugs that are substrates of CYP2D6 and are detoxicated by this enzyme, such as 4-methoxyamphetamine (Kitchen *et al.*, 1979).

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