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Research Paper

Metabolic interactions with piperazine-based 'party pill' drugs

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Abstract

Objectives 'Party pills' have found use worldwide as a substitute for amphetamine-derived designer drugs. Whilst some information exists about the metabolism of these drugs, there is little information about their ability to inhibit the metabolism of co-administered drugs. This study aimed to determine whether predictions can be made about global interactions between 'party pills' constituents and other drugs metabolised by the same cytochrome P450 (CYP) isoenzymes.

Methods The inhibitory effects of seven benzyl and phenyl piperazines were measured in microsomal incubation assays of probe substrates for five major CYP isoenzymes. In addition, the metabolism of benzylpiperazine and trifluoromethylphenylpiperazine, the two most commonly used constituents of 'party pills', was investigated using human liver microsomes assays and known inhibitors of CYP isoenzymes.

Key findings All piperazine analogues tested showed significant inhibitory activity against most, if not all, isoenzymes tested. The metabolism of benzylpiperazine (BZP) and trifluoromethylphenylpiperazine (TFMPP) involved CYP2D6, CYP1A2 and CYP3A4. Furthermore, BZP and TFMPP inhibited each other's metabolism.

Conclusions Fluorophenylpiperazine, methoxyphenylpiperazine, chlorophenylpiperazine, methylbenzylpiperazine and methylenedioxybenzylpiperazine had significant inhibitory effects on CYP2D6, CYP1A2, CYP3A4, CYP2C19 and CYP2C9 isoenzymes but each piperazine had a different inhibitory profile. The metabolic interaction between BZP and TFMPP may have clinical implications, as these agents are often combined in 'party pills'.

Keywords cytochrome P450 isoenzymes; benzylpiperazine; drug–drug interactions; trifluoromethylphenylpiperazine; piperazine analogues

Introduction

Piperazine-based compounds such as trifluoromethylphenylpiperazine (TFMPP), benzylpiperazine (BZP), methoxyphenylpiperazine (MeOPP), chlorophenylpiperazine (mCPP), fluorophenylpiperazine (pFPP), methylbenzylpiperazine (MBZP) and methylenedioxybenzylpiperazine (MDBP) (Figure 1) are widely used around the world as recreational drugs. BZP and TFMPP are the most commonly encountered active constituents of 'party pills' or 'herbal highs'; however, because of the legislation surrounding these compounds, the popularity of other piperazine analogues is increasing. 'Party pills' are frequently sold over the internet and often have varying types and amounts of the active ingredients, with names like 'Charge' (50 mg BZP and 200 mg TFMPP), 'Bliss' (100 mg BZP and 50 mg TFMPP) or 'Mash' (37.5 mg pFPP). The subjective effects of BZP and TFMPP have been reported to mimic the actions of the stimulant amphetamine [1] and the hallucinogen lysergic acid (LSD),[2] respectively, while combinations of these drugs result in effects similar to those of 3,4-methylenedioxymethamphetamine (MDMA; 'ecstasy').[3]

Since the late 1990s, a number of studies have sought to fill the gap in knowledge surrounding these compounds. Previous studies have indicated the importance of cytochrome P450 (CYP) isozymes CYP2D6, CYP1A2 and CYP3A4 in the metabolism of TFMPP,[4] and preliminary data on the inhibitory effects of BZP and TFMPP on these enzymes have already been reported.[5] Several studies have also described the metabolism of MeOPP,[6] MDBP,[7] mCPP[8,9] and pFPP,[10] again implicating major P450 enzymes in the hydroxylation and/or de-methylation of these drugs.

The aim of this study was to investigate the effect of BZP, TFMPP, MeOPP, mCPP, pFPP, MBZP and MDBP on CYP2D6, CYP1A2, CYP3A4, CYP2C9 and CYP2C19.

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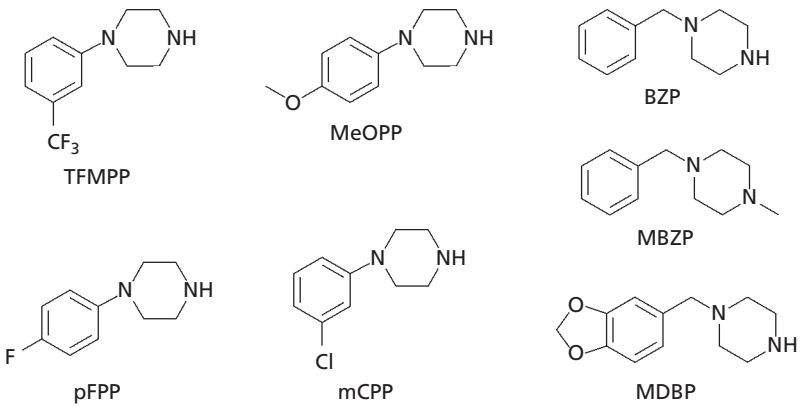

Figure 1 Chemical structures of piperazine-based drugs commonly encountered in 'party pill' preparations. TFMPP, trifluoromethylphenylpiperazine; MeOPP, methoxyphenylpiperazine; BZP, benzylpiperazine; pFPP, fluorophenylpiperazine; mCPP, chlorophenylpiperazine; MBZP, methylbenzylpiperazine; MDBP, methylenedioxybenzylpiperazine.

Drug–drug interactions (DDIs) have a wide variety of consequences, ranging from no clinical significance to a lack of therapeutic efficacy or severe toxicity and fatalities. Studies have demonstrated that DDIs lead to increased rates of hospitalisations and length of stay and associated healthcare costs.[11] These issues are important to consider when prescribing medicines.[12] DDIs are also particularly relevant in users of 'party pills'. Concomitant use of other substances, including methylphenidate (Ritalin), MDMA and LSD, has been reported to cause severe adverse reactions.[13,14] Co-administration of BZP and TFMPP has been shown to lead to seizures in rats[15] and nausea and other symptoms of toxicity in humans,[16] hence, the importance of conducting research to determine the metabolism of these drugs and investigate potential interactions.

Materials and Methods

Materials

BZP, TFMPP, dextromethorphan, caffeine, ethinylestradiol, omeprazole, tolbutamide, quinidine, troleandomycin, tetrahydrofuran, perchloric acid, phenytoin, sodium dihydrogen phosphate, TFMPP, BZP, MBZP, MDBP, MeOPP, pFPP and mCPP were purchased from Sigma-Aldrich, St Louis, MO, USA. NADPH was obtained from Applichem, Darmstadt, Germany. Acetonitrile and methanol were from Scharlau Chemie, Sentmenat, Spain. Sulfaphenazole was obtained from Ciba-Geigy, Basle, Switzerland. Proguanil was purchased from AstraZeneca, Alderley Edge, UK; furafylline was from Ultrafine Chemicals, Manchester, UK. Ammonium formate was from Acros Organics, NJ, USA.

Microsomal incubation

All incubations had a final volume of 100 μl, adjusted using phosphate buffer (67 mM, pH 7.4). This study used pooled human liver microsomes rather than single-donor microsomes to minimise the effect of inter-individual variability. Liver samples were from three male and three female Caucasian non-smokers (age range 29–73 years) and were collected by liver resection for colorectal cancer metastasis. The tissue samples were histologically normal. The livers have been shown to express many CYP enzymes, including those studied, by Western blotting and have shown drug metabolising capacity similar to literature values.

Incubation times and protein concentrations were optimised to achieve 10–15% metabolism of the substrate. These concentrations aim to maintain the selectivity of the isoform-specific substrate, as most substrates lose their selectivity at high concentrations.[17] Inhibitor concentrations for positive and negative control inhibitors were consistent with their inhibition constants (k_i), shown in Table 1.

Inhibition of CYPs by piperazine drugs

Experiments were conducted to investigate the inhibitory effects of piperzines on the metabolism of probe substrates dextromethorphan (CYP2D6), ethinylestradiol (CYP3A4),

Table 1 Substrates and controls used in inhibition assays. A substrate concentration of 200 μM was used in all incubations. Concentrations for positive control inhibitors are consistent with their k_i values

Enzyme	Probe substrate	Positive control inhibitor	Negative control inhibitor
CYP2D6	Dextromethorphan	Quinidine (25 μM)	Furafylline (50 μM)
CYP1A2	Caffeine	Furafylline (50 μM)	Quinidine (25 μM)
CYP3A4	Ethinylestradiol	Troleandomycin (500 μM)	Furafylline (50 μM)
CYP2C19	Omeprazole	Proguanil (100 μM)	Furafylline (50 μM)
CYP2C9	Tolbutamide	Sulfaphenazole (0.5 μM)	Furafylline (50 μM)

Positive control inhibitors are known inhibitors of the enzyme in question; negative control inhibitors inhibit other CYP isoforms.

caffeine (CYP1A2), omeprazole (CYP2C19) and tolbutamide (CYP2C9).

For each enzyme, incubations were performed with each of the following piperazines: BZP, TFMPP, pFPP, MeOPP, mCPP, MBZP and MDBP. Incubations containing no inhibitors (volume replaced by phosphate buffer), negative control inhibitors (an inhibitor of an enzyme other than the one being probed) and a positive control inhibitor (a known inhibitor of the enzyme being probed) were used to validate results.

Incubation mixtures ($n = 6$) contained pooled liver microsomes (40 μg), NADPH (2 mM), a piperazine-based drug (200 μM) and phosphate buffer (67 mM, pH 7.4). Samples were pre-incubated for 5 min in a 37°C water bath before the addition of probe substrate (200 μM; see Table 1 for details of probe substrates). Samples were then incubated for 30 min at 37°C.

Metabolism of BZP and TFMPP

Further incubations were carried out to investigate the effect of known CYP inhibitors on the metabolism of BZP and TFMPP. These incubation mixtures ($n = 6$) contained pooled liver microsomes (40 μg), NADPH (2 mM), a known CYP inhibitor (see Table 2 for inhibitors and their concentrations) and phosphate buffer (67 mM, pH 7.4). The samples were pre-incubated for 5 min in a 37°C water bath before the addition of BZP or TFMPP (200 μM). Samples were then incubated for 30 min at 37°C.

Interaction between BZP and TFMPP

Further incubations to characterise interactions between BZP and TFMPP ($n = 6$) were conducted containing pooled liver microsomes (40 μg), NADPH (2 mM) and phosphate buffer (67 mM, pH 7.4). Samples were pre-incubated for 5 min at 37°C before the addition of BZP and TFMPP (200 μM). Samples were then incubated for 30 min at 37°C.

Termination and clean-up of incubations

Dextromethorphan, caffeine, ethinylestradiol, BZP and TFMPP incubations were terminated by addition of 37% perchloric acid (5 μl). Because of the instability of omeprazole and tolbutamide under acidic conditions, these incubations were terminated by the addition of ice-cold acetonitrile (100 μl). Samples were mixed thoroughly and stored at −20°C for 60 min to ensure precipitation of the protein. Following this, samples were thawed and then centrifuged for 10 min at 10 000 rpm. An aliquot of supernatant (80 μl) was used for analysis.

Analysis

All samples were analysed using HPLC with UV detection. Separation was conducted using an Agilent series 1100 HPLC system with an Agilent series 1100 multiple wavelength detector and an Agilent Extend reverse-phase C_{18} column (150 × 4.6 mm, 5 μm) (Santa Clara, CA, USA). Table 3 shows the optimal detection wavelengths used for each analyte and their retention times.

For the BZP incubations, where increased sensitivity was required, liquid chromatography with mass spectrometry (LC–MS) on an Agilent MSD model D single-staged quadrupole mass spectrum detector was used.

Standards for microsomes, NADPH, negative control, positive control and each piperazine drug were analysed by HPLC and no interference was observed between the components of each incubation. Standards of the substrates used in each experiment were prepared in triplicate at concentrations ranging from 100 μM to 200 μM. This data was used to construct standard curves. The linearity, accuracy and precision of each method are shown in Table 3.

Dextromethorphan

The mobile phase consisted of 10 mM formate buffer, pH 4.5, and acetonitrile, delivered at a flow rate of 1 ml/min. The solvent ratio (formate buffer : acetonitrile) was 90 : 10 at 0–15 min, 20 : 80 at 15–17 min and 90 : 10 at 17–20 min.

Caffeine

The aqueous mobile phase consisted of 10 mM formate buffer, pH 4 0, with 1% methanol, 1% acetonitrile and 1.6% tetrahydrofuran added. The organic mobile phase was acetonitrile. The flow rate of the mobile phase was 1 ml/min. The solvent ratio (aqueous : organic) was 100 : 0 at 0–12.5 min, 20 : 80 at 12.5–15.5 min and 100 : 0 at 15.5–20 min.

Ethinylestradiol

The mobile phase consisted of MilliQ water and acetonitrile, delivered at a flow rate of 1 ml/min. The solvent ratio (MilliQ water : acetonitrile) was 55 : 45 at 0–10 min, 20 : 80 at 10–18.5 min, and 55 : 45 at 18.5–20 min.

Table 2 Effect of specific enzyme inhibitors on the metabolism of benzylpiperazine and trifluoromethylphenylpiperazine (both at 200 μM)

Enzyme	Inhibitor	BZP	TFMPP
Control	None (buffer only)	15 452 (± 680)	1184 (± 312)
CYP2D6	Quinidine (25 μM)	4255 (± 346)**	197 (± 40)**
CYP1A2	Furafylline (50 μM)	11 449 (± 684)**	754 (± 26)**
CYP3A4	Troleandomycin (100 μM)	8328 (± 520)**	968 (± 10)*
CYP2C9	Proguanil (500 μM)	13 677 (± 583)	1191 (± 88)
CYP2C19	Phenytoin (500 μM)	13 361 (± 597)	1190 (± 70)

Values are substrate turnover (mean ± SD, $n = 6$) in nmol/min per mg protein. *$P < 0.05$; **$P < 0.01$ vs 'no inhibitor' control. BZP, benzylpiperazine; TFMPP, trifluoromethylphenylpiperazine.

Table 3 Optimal detection wavelengths, retention times, calculated linearity, precision and accuracy for each substrate assay ($n = 6$)

Analyte	Wavelength (nm)	Retention time (min)	Linearity (R^2)	Precision (%)	Accuracy (%)
Dextromethorphan	231	11.3	0.97	1.9	98.20
Caffeine	224	4.8	0.99	2.1	91.46
Ethinylestradiol	212	3.6	0.99	3.6	98.74
Omeprazole	302	6.7	0.95	6.1	95.69
Tolbutamide	230	7.1	0.99	3.9	94.57
TFMPP	256	9.2	0.99	2.2	96.99
BZP	*m/z* 177[a]	7.6	0.99	3.7	93.08

BZP, benzylpiperazine; TFMPP, trifluoromethylphenylpiperazine. [a]Single-ion monitoring.

Omeprazole

The mobile phase consisted of 70% 50 mM phosphate buffer, pH 8.0, and 30% acetonitrile, delivered at a flow rate of 1.1 ml/min.

Tolbutamide and CYP2C9

The mobile phase consisted of 0.5% ammonium acetate in MilliQ water and 0.5% ammonium acetate in methanol, delivered at a flow rate of 1 ml/min. The solvent ratio (ammonium acetate in MilliQ water : ammonium acetate in methanol) was 70 : 30 at 0–10 min, 5 : 95 at 10–12 min and 70 : 30 at 12–20 minutes.

TFMPP and BZP

The mobile phase consisted of 10 mM formate buffer, pH 4.5, and acetonitrile, delivered at a flow rate of 1 ml/min. A phase gradient with the following % acetonitrile was used for separation: 0–2 min 5%; 2–5 min 10%; 5–10 min 10–55%; 10–12 min 55–5%; 12–15 min 5%. Detection by single-ion monitoring by MS was used for BZP because of its weak UV absorbance.

Statistical analysis

The substrate turnover (nmol/min per mg protein) was calculated from the loss of parent drug (nmol), incubation time (min) and amount of protein (mg) for all incubations.

Differences in the metabolism of substrate between samples with and without inhibitor were compared by single-factor analysis of variance (ANOVA). P values less than 0.05 were considered statistically significant.

Results

TFMPP and MBZP inhibited the metabolism of all probe substrates analysed. All of the piperazines except for BZP inhibited CYP2C19 (Table 4). No generalisations could be made across the classes of piperazines.

The metabolism of BZP and TFMPP was significantly inhibited by the inhibitors of CYP2D6, CYP1A2 and CYP3A4, but not by inhibitors of CYP2C9 and CYP2C19 (Table 2).

BZP and TFMPP also significantly inhibited each others' metabolism ($P < 0.0001$). TFMPP inhibited the metabolism of BZP by nearly 60% (turnover of BZP decreased from

Table 4 Inhibitory action of benzylpiperazines and phenylpiperazines on probe substrates of CYP isoenzymes

	CYP2D6	CYP1A2	CYP3A4	CYP2C19	CYP2C9
Substrate	Dextromethorphan	Caffeine	Ethinylestradiol	Omeprazole	Tolbutamide
No inhibitor	21 941 ± 5380	15 790 ± 5643	101 537 ± 24 055	175 696 ± 15 879	129 717 ± 18 546
Positive control inhibitor	12 726 ± 1598*	1016 ± 885†	44 677 ± 8561.3*	54 165 ± 23 075†	90 259 ± 7448**
Negative control inhibitor	17 756 ± 4057	12 461 ± 5023	128 421 ± 26 837	177 820 ± 43 556	131 927 ± 14 519
Benzylpiperazines					
MBZP	7034 ± 2573*	6149 ± 3217**	61 250 ± 8323*	106 553 ± 43 784†	96 816 ± 4204**
MDBP	6232 ± 5469	1805 ± 1634†	68 549 ± 20 794	54 997 ± 29 642†	68 088 ± 7664*
BZP	15 512 ± 155†	2968 ± 90†	89 454 ± 805†	176 712 ± 601	67 897 ± 18 776**
Phenylpiperazines					
TFMPP	8405 ± 874*	4195 ± 1625†	65 386 ± 9560**	148 687 ± 19 956*	83 541 ± 20 796**
MeOPP	6379 ± 1429†	15 243 ± 4135	33 319 ± 6667**	80 539 ± 25 293*	48 671 ± 17 204†
pFPP	19 953 ± 4804	4309 ± 1136**	50 317 ± 4659**	115 344 ± 26 020*	118 746 ± 21 749
mCPP	16 018 ± 1987	3273 ± 1370†	81 902 ± 26 606	138 957 ± 28 713*	58 957 ± 35 182**

Concentration of probe substrates and piperazine-based drugs was 200 μM for all incubations. Positive control inhibitors are known inhibitors of the enzyme in question; negative control inhibitors inhibit other CYP isoforms. Values are substrate turnover (mean ± SD, $n = 6$) in nmol/min/mg protein. *$P < 0.05$; **$P < 0.01$; $^{\dagger}P < 0.001$ vs 'no inhibitor' control. MBZP, methylbenzylpiperazine; MDBP, methylenedioxybenzylpiperazine; BZP, benzylpiperazine; TFMPP, trifluoromethylphenylpiperazine; MeOPP, methoxyphenylpiperazine; pFPP, fluorophenylpiperazine; mCPP, chlorophenylpiperazine.

15 452 (± 680) to 6178 (± 267) nmol/min per mg protein in the presence of TFMPP ($P < 0.001$). BZP also inhibited the metabolism of TFMPP by approximately 91%. (Turnover of TFMPP decreased from 1184 (± 312) to 107 (± 323) nmol/min per mg protein in the presence of BZP ($P < 0.001$).

Positive controls showed significant inhibition, while negative controls exhibited no significant inhibition, thereby confirming the validity of these results.

Discussion

A significant decrease in the metabolism of a probe substrate in the presence of an inhibitor (compared with the 'no inhibitor' or negative controls) was assumed to be indicative of inhibition of the CYP isoenzyme involved, while no significant difference indicates no enzyme inhibition. However, this may not always be the case, as factors such as protein binding (of the substrate or inhibitor) and complexation (substrate and inhibitor) can confound the validity of the results. Furthermore, some probe substrates have the potential to inhibit other enzymes, or may be substrates of other enzymes. This is true for dextromethorphan and omeprazole, which are metabolised by CYP3A4 to a small extent. Such interferences were minimised by maintaining a low level of substrate turnover (10–15%); nevertheless, this still remains a potential limitation of this study.

While it has been shown that these piperazines are likely to inhibit the metabolism of many commonly used drugs, it cannot be determined from these data alone whether the inhibition observed is competitive or non-competitive. MDMA inhibits CYP2D6 because of high affinity of its methylenedioxy moiety for the CYP2D6 enzyme;[18] it is therefore possible that a similar action accounts for the inhibition of this enzyme by MDBP, which has the same functional group.

The k_i of each piperazine-based drug has not been determined in this study, but some comparative estimates of k_i can be made. The extent of inhibition can be estimated by comparing the amount of inhibition (denoted as substrate turnover in Table 4) between the positive controls and incubations with piperazine-based drugs. For example, when compared with the effects of quinidine in the dextromethorphan (CYP2D6) incubations, BZP, MeOPP and TFMPP caused significantly more inhibition, TFMPP and MBZP showed similar amounts of inhibition, and pFPP and mCPP showed little or no inhibition. Rough estimates of k_i of each of the piperazines can be gleaned from these data (i.e. less than, approximately equal to, or significantly greater than 25 μM (k_i of quinidine) for CYP2D6).

When the metabolism of BZP and TFMPP was investigated, the results indicate that BZP and TFMPP inhibit more enzymes (Table 4) than they themselves are metabolised by (Table 2). As this could be the case for other piperazines, further studies are required in order to fully understand their metabolism.

An important and worrying finding is the inhibition of BZP and TFMPP metabolism when these two drugs are co-incubated, as 'party pill' formulations often contain both these drugs. A metabolic interaction between these drugs can lead to elevated levels of both drugs and enhanced effects and increased incidence of adverse events.

This study has a few limitations which future studies may be able to address. Firstly, other enzymes could be involved in the metabolism of, or be inhibited by, the piperazine-based drugs. One such enzyme is CYP2B6 which has been shown to be involved in the metabolism of nicotine,[19] and is suggested to be involved in the metabolism of amphetamine and cocaine in the brain.[20] Secondly, the substrate concentration used for all incubations was set at 200 μM, which is higher than plasma concentrations reported in recent studies (approximately 1 μM for BZP in a human study[21]). However, a recent animal study reported that ratios of plasma to hepatic concentrations of these drugs were approximately 1 : 5 for BZP and 1 : 50 for TFMPP.[22] This recent information must be considered when designing future in-vitro studies.

Conclusions

This research is timely, as it is likely that the criminalisation of piperazine-based drugs will result in them entering the illicit drug market. This is the first study showing the inhibitory effects of BZP, TFMPP, pFPP, MeOPP, mCPP, MBZP and MDBP on the CYP2D6, CYP1A2, CYP3A4, CYP2C19 and CYP2C9 isoenzymes.

This in-vitro study indicates that there is a potential for interactions between piperazine-based drugs and other drugs. This information is clinically relevant as 'party pill' users may be taking other medicines that are metabolised by the same cytochrome isoforms; for example paroxetine is also metabolised by CYP2D6;[23] erythromycin is metabolised by CYP3A4.[24] This could lead to potential DDIs, resulting in loss of therapeutic efficacy, or toxicity. Furthermore, co-administration of several recreational drugs (including MDMA and LSD) with 'party pill' drugs often results in adverse drug reactions.[14]

These results also show that significant interactions may arise between two or more co-administered piperazine-based drugs. As piperazine-based drugs are combined in 'party pill' formulations, this finding has important clinical ramifications. The reported adverse effects of combining BZP and TFMPP[15,16] further indicate the clinical relevance of these interactions. A recent human study has also indicated that significant pharmacokinetic interactions are seen between BZP and TFMPP.[25] The results of this study also emphasise the importance of studying these piperazines individually.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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