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Role of CYP3A in bromperidol metabolism in rat *in vitro* and *in vivo*

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1. The aim was to identify whether CYP3A metabolizes bromperidol (BP), an antipsychotic drug, to form 4-fluorobenzoyl-propionic acid (FBPA) in hepatic microsomes from 8-week-old male Sprague–Dawley rats and to investigate whether an inhibitor or an inducer of CYP3A affects BP pharmacokinetics in rat.

2. In an *in vitro* study, only troleandomycin showed marked inhibition of FBPA formation among several specific CYP isozyme inhibitors studied including troleandomycin, diethyldithiocarbamate, furafylline and quinine. Anti-rat CYP3A2 serum inhibited FBPA formation by 80%, whereas other anti-rat CYP sera (1A1, 1A2, 2B1, 2C11, 2E1) only slightly inhibited it.

3. In a pharmacokinetic study, BP half-life was prolonged to 137% of the average control value by 7-day treatment with erythromycin, a CYP3A inhibitor, and shortened to 58% of the control by 2-day treatment with dexamethasone, a CYP3A inducer. BP clearance was reduced to 68% of the control by erythromycin and was increased to 145% of control by dexamethasone.

4. These results suggested that BP biotransformation is catalysed mainly by CYP3A to form FBPA in rat and that the modification of this enzyme activity would affect the pharmacokinetics of BP.

Introduction

Bromperidol (BP), a butyrophenone-typeneuroleptic, is used in the treatment of patients with psychiatric disease, similarly to haloperidol (HP). Although BP is a close structural analogue of HP, with the only difference being bromine in BP in place of chlorine in HP, some differences have been found in their treatment effects on specific symptoms in studies of patients with chronic schizophrenia. Malfroid *et al.* (1978) reported that BP has a more pronounced anxiolytic effect than HP and Itoh (1985) found that BP was superior to HP in improving schizophrenic symptoms in a double-blind controlled study. In addition, BP is reported to have a faster onset of action than HP (Itoh 1985, Benfield *et al.* 1988). The incidence of extrapyramidal side effects was similar after both treatments (Malfroid *et al.* 1978, Denjis 1980, Brannen *et al.* 1981). Therefore, BP is considered as useful as HP in the treatment of patients with psychiatric disease.

Previous studies suggest that BP undergoes three metabolic pathways: *O*-glucuronide conjugation, carbonyl reduction and oxidative *N*-dealkylation (Chasseaud 1978, Heykants *et al.* 1978, Wong *et al.* 1983, Someya *et al.* 1991). Although HP is reported to be *N*-dealkylated by CYP3A (Fang *et al.* 1997, Pan *et al.* 1997), the isoenzymes catalysing the metabolism of BP have not been clarified. Carbamazepine, an inducer of CYP3A, significantly decreased plasma

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concentrations of BP in a previous clinical study (Otani *et al*. 1997), which suggests that BP is *N*-dealkylated by CYP3A and that the modification of this enzyme activity by an inhibitor or an inducer may affect BP pharmacokinetics. The present authors, therefore, studied whether CYP3A catalysed BP *N*-dealkylation and whether the pharmacokinetics of BP was actually affected when the CYP3A activity in liver microsomes was altered by an inhibitor or an inducer of CYP3A in rat.

Materials and methods

In vitro *experiments*

Preparation of liver microsomes. Male Sprague–Dawley rats ($n = 10$, body weight of 272–305 g) were purchased from Nippon Clea (Tokyo, Japan). Animals were killed by decapitation, and the livers were immediately removed and homogenized with 3 vols 1.15% KCl. Hepatic microsomes were prepared by sequential centrifugation of the homogenate at 9000*g* for 20 min and of the resultant supernatant at 105000*g* for 60 min, both at 4 °C. The precipitates were mixed and diluted with distilled water (about 10 mg/ml). The microsome samples were separated into approximately 200 tubes, immediately frozen in liquid nitrogen, and stored at − 80 °C until use. Protein concentrations were determined by the method of Lowry *et al*. (1951).

Chemicals. The chemicals used were: $NADP^+$, glucose 6-phosphate and glucose 6-phosphate dehydrogenase (Oriental Yeast, Tokyo, Japan); HP, BP and reduced BP (RBP) (Yoshitomi Pharmaceutical Co., Ltd, Osaka, Japan); 4-fluorobenzoyl-propionicacid (FBPA) and SKF-525A (Research Biochemicals International, Natick, MA, USA); troleandomycin and quinine (Sigma Chemical Co., St Louis, MO, USA); diethyldithiocarbamate (DDC) (Wako Pure Chemical Industries, Osaka, Japan). All other chemicals were purchased from Wako Pure Chemical Industries and were of analytical grade.

Incubation conditions. The incubation mixture used to assay the formation of FBPA from BP consisted of 100 mM Na-K phosphate buffer (pH 7.4), 0.05 mM EDTA, 0.8 mg microsomal protein, and a substrate (100 μM BP). The reaction was started by the addition of an NADPH-generating system (5 mM magnesium chloride, 0.5 mM $NADP^+$, 5 mM glucose 6-phosphate and 1 unit of glucose 6-phosphate dehydrogenase) in a final volume of 1 ml. After incubation for 30 min at 37 °C, the reaction was terminated by the addition of 1 ml acetonitrile. The incubation (30 min) and amount of microsomal protein (0.8 mg) were determined by results showing that FBPA formation rate increased linearly in a range of 0.4–2.0 mg microsomal protein and 15–60 min of incubation.

FBPA measurement. A solution of 20 nmol HP (internal standard) in 100 μl methanol was added to each mixture. The denatured proteins were removed by centrifugation, and 50 μl 2 N HCl was added to 1.5 ml supernatant. The mixture was extracted with 5 ml dichloromethane for 30 s on a vortex mixer. After centrifugation, the organic layer was separated and evaporated. The residue was reconstituted with 300 μl of the HPLC mobile phase, and 100 μl was injected into the chromatographic system. BP, RBP, FBPA and HP were measured in the reaction medium by high-performance liquid chromatography (HPLC) using a slightly modified method of Fang and Gorrod (1993). Briefly, the chromatographic system comprised of a CCPM-II solvent pump (TOSOH, Tokyo, Japan) and a MCPD-3600 spectro-multi-channel photo detector (OTUKA ELECTRONICS, Osaka, Japan) set to measure at 220 and 245 nm wavelengths. The HPLC system used a 5 μm TSK CN-80Ts column (silica gel with bonded cyanopropyl groups, 250 × 4.6 mm i.d.) (TOSOH, Tokyo, Japan). Mobile phase A(acetonitrile) and mobile phase B (30 mM ammonium acetate buffer adjusted to pH 5.0 with acetic acid) were used in a gradient program at 1.2 ml/min: A was 20% at 0–1 min, 20–30% linearly from 1 to 15 min, and 20% at 15–25 min.

Inhibition experiments. The effect of various specific and non-specific inhibitors of CYP on the rate of FBPA production from BP were examined to identify the CYP isozyme(s) that catalysed BP *N*-de-alkylation. Troleandomycin (a CYP3A inhibitor), diethyldithiocarbamate (a CYP2E1 inhibitor), furafylline (a CYP1A2 inhibitor), quinine (a CYP2D inhibitor) or SKF-525A were added to the incubation mixture as a specific or non-specific inhibitor of a CYP isozyme at 20, 40 and 100 μM (2, 4 and 10 μM for quinine). Troleandomycin, diethyldithiocarbamate, furafylline, quinine and SKF-525A were pre-incubated with an NADPH-generating system for 10, 3, 10, 2 and 5 min respectively before the reaction was started (Schenkman *et al*. 1972, Kobayashi *et al*. 1989, Kunze and Trager 1993, Bourrie *et al*. 1996, Nunoya *et al*. 1996). Also examined was the inhibitory effect of quinine on dextromethorphan *O*-demethylation activity (Kerry *et al*. 1993) and it was compared with the effect on BP *N*-dealkylation.

Immuno-inhibition experiments. Anti-rat CYP1A1, 1A2, 2B1, 2C11, 2E1 and 3A2 antibodies were purchased from Daiichi Pure Chemicals Co., Ltd (Tokyo, Japan). The immuno-inhibition of the FBPA formation was examined by pre-incubation of rats liver microsomes (0.8 mg) with various anti-rat CYP sera (0.2 mg IgG) at room temperature for 30 min before the reaction was started with 100 μM BP and an NADPH-generating system. Individual pre-immune sera were pre-incubated under the same conditions as a control for the various anti-rat CYP sera.

In vivo *experiments*

Animals and procedures. The treatment regiment consisted of either erythromycin (100 mg/kg per day) or dexamethasone (80 mg/kg per day) suspended in corn oil and administered to male Sprague–Dawley rats ($n = 6$ in each treatment group, with body weight of 273–308 g) by intraperitoneal injection (i.p.) for 7 or 2 days respectively during the eighth week of age. The respective treatment regimens were chosen because the 7-day treatment with 100 mg/kg erythromycin has previously been shown significantly to increase the serum levels of cyclosporin, a substrate for CYP3A, in rat (Murray *et al.* 1992) and because the 2-day treatment with 80 mg/kg dexamethasone has been shown significantly to induce CYP3A in hepatic microsomes (Kolars *et al.* 1992). Corn oil vehicle was administered at the same time to another group ($n = 6$) serving as control. Rats received a single intravenous injection of 0.5 mg/kg BP (Serenace Injection, Dainippon Pharmaceutical Co., Ltd, Osaka, Japan) in the tail vein 1 h after the last administration of erythromycin or corn oil and 24 h after the last administration of dexamethasone.

Determination of BP and pharmacokinetic parameters. At 0.25, 0.5, 1, 2, 4, 6 and 8 h after injection of BP, blood was collected from the tail vein on the reverse side of the one used for injection. The concentration of BP in plasma was measured by enzyme immunoassay (MARKIT-M Bromperidol, Dainippon). Pharmacokinetic parameters for BP were obtained from plasma concentration–time profiles. Area under the curve (AUC) was determined by the trapezoidal rule. The values were extrapolated to infinity by dividing the last measured serum concentration by β, the slope of the terminal elimination phase (BP concentration at 2, 4, 6 and 8 h after the administration) obtained by least-squares linear regression. $t_{1/2}$ was calculated as $t_{1/2} = \ln 2/\beta$. The volume of distribution (V_d) and clearance (Cl) were determined by the following respective ratios: dose/(AUC $\times$ β) and dose/AUC.

Statistical analysis

Results of the *in vitro* studies are expressed as means from duplicate experiments, and results of the *in vivo* studies are expressed as mean $\pm$ SEM. Means of the latter were compared with one-way analysis of variance followed by Scheffe test. $p < 0.05$ was considered as statistically significant.

Results

In vitro *study*

Figure 1 shows a chromatogram of 1.25 nmol of the standard compounds including FBPA, HP, BP and RBP. FBPA formation followed Michaelis–Menten kinetics and the K_m and V_{max} were 18.9 μM and 333.0 pmol/min/mg of protein respectively.

The reaction resulting in FBPA formation from BP (BP *N*-dealkylation activity) was inhibited by 100 μM SKF-525A to 6.9% of the control and was minimal in the absence of an NADPH-generating system (data not shown). Figure 2 shows the effect of four chemical inhibitors on BP *N*-dealkylation activity. Troleandomycin (20 μM) inhibited BP *N*-dealkylation to 38% of the control, whereas 100 μM DDC or furafylline only marginally inhibited the *N*-dealkylation. Of quinine, which reduced dextromethorphan *O*-demethylation to 44% of the control, 2 μM slightly inhibited BP *N*-dealkylation to 82% of the control.

Figure 3 shows the effect of six anti-rat CYP sera on BP *N*-dealkylation activity. Anti-rat CYP3A2 serum showed an 80% decrease in BP *N*-dealkylation activity, whereas the other anti-rat CYP sera could not inhibit activity.

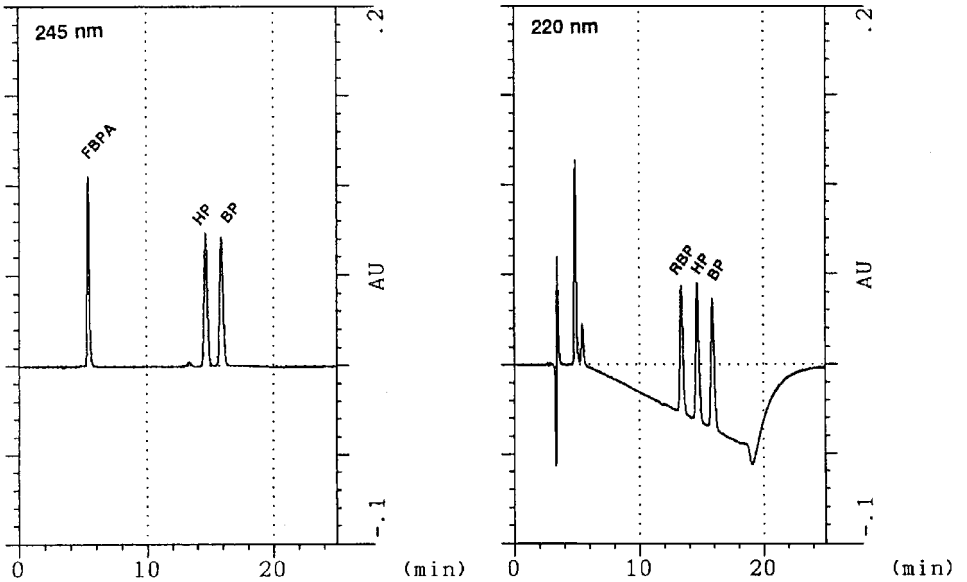

Figure 1. Chromatogram of a mixture of standard compounds (1.25 nmol each). HP, haloperidol; BP, bromperidol; RBP, reduced bromperidol; FBPA; 4-fluorobenzoylpropionic acid. The left panel was monitored at 245 nm; the right at 220 nm.

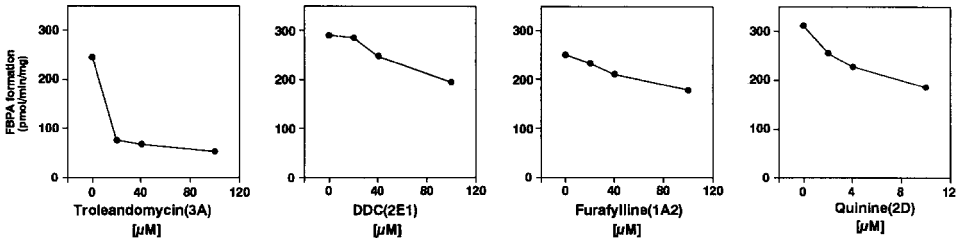

Figure 2. Effects of various specific cytochrome P450 inhibitors on 4-fluorobenzoylpropionic acid (FBPA) formation in the hepatic microsomes of rat. Each point represents the mean of duplicate determinations.

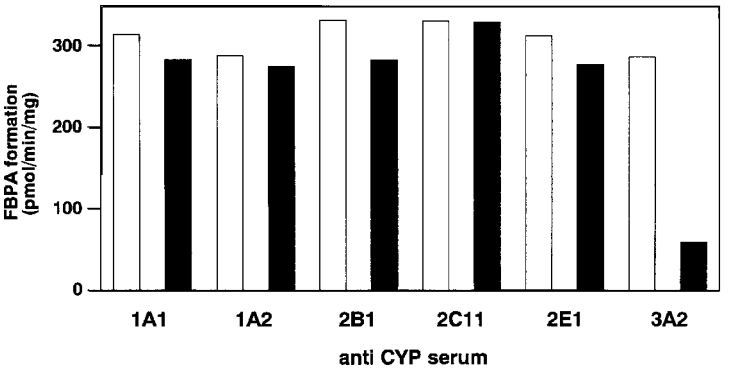

Figure 3. Effects of various anti-CYP serum on 4-fluorobenzoylpropionic acid (FBPA) formation in the hepatic microsomes of rat. The immuno-inhibition of the FBPA formation was examined by pre-incubation of rat liver microsomes (0.8 mg) with various anti-rat CYP sera (■) at room temperature for 30 min before the reaction was started. Individual pre-immune serum (□) was pre-incubated under the same conditions as a control for the various anti-rat CYP sera. Each value represents the mean of duplicate determinations.

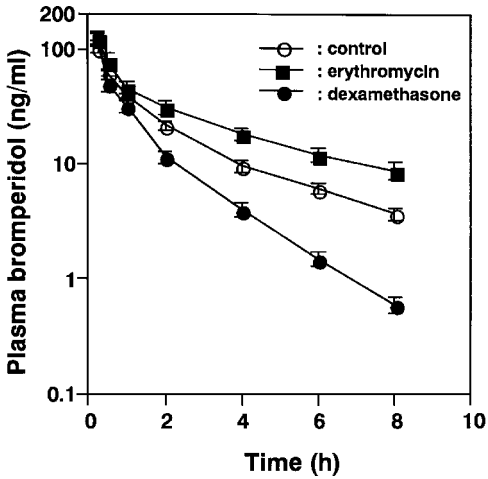

Figure 4. Effect of erythromycin and dexamethasone on plasma bromperidol concentration. Six male Sprague–Dawley rats were pretreated intraperitoneally with either erythromycin (100 mg/kg per day for 7 days, ■) or dexamethasone (80 mg/kg per day for 2 days, ●) as a suspension in corn oil. The corn oil vehicle alone (○) was administered at the same condition as to the control group ($n = 6$). Rats received a single intravenous injection of 0.5 mg/kg bromperidol in the tail vein.

Table 1. Effect of erythromycin and dexamethasone on the pharmacokinetic parameters of bromperidol.

	$t_{1/2}$ (h)	AUC (ng·h/ml)	*Cl* (ml/h/kg)	V_d (ml/kg)
Control ($n = 6$)	2.43±0.12	176.7±11.5	2.89±0.20	10.3±1.0
Erythromycin ($n = 6$)	3.34±0.20*	274.4±38.1*	1.95±0.19	9.3±1.0
Dexamethasone ($n = 6$)	1.42±0.08$^{\#\#}$	127.2±13.8$^{\#}$	4.20±0.51$^{\#}$	8.6±1.1

Pharmacokinetic parameters for bromperidol were obtained from plasma concentration–time profiles (figure 4).

$t_{1/2}$, elimination half-life; AUC, area under the plasma bromperidol concentration; *Cl*, total body clearance; V_d, volume of distribution.

*$p < 0.05$, **$p < 0.01$, significantly different from control.

$^{\#}p < 0.01$, $^{\#\#}p < 0.001$, significantly different from erythromycin.

Pharmacokinetic study

Plasma BP concentration–time profiles are shown in figure 4; pharmacokinetic parameters obtained from the profiles are summarized in table 1. The $t_{1/2}$ and AUC for BP were increased by erythromycin pretreatment to 137 and 155% of the control, respectively and were reduced by dexamethasone pretreatment to 58 and 72% of the control respectively. Clearance of BP was reduced by erythromycin pretreatment to 68% of the control and increased by dexamethasone pretreatment to 145% of the control. These parameters were significantly different between erythromycin and dexamethasone pretreatment groups.

Discussion

In the present study, CYP3A catalysed BP to FBPA in hepatic microsomes of rat, and an inducer and an inhibitor of CYP3A altered BP pharmacokinetics in rat. FBPA production was inhibited by SKF-525A (a non-specific inhibitor of

CYP), and FBPA was hardly detected in the absence of an NADPH-generating system, both of which suggest that one or more members of the CYP subfamilies *N*-dealkylate(s) BP to FBPA. The effects of various chemical inhibitors and antibodies on *N*-dealkylation activity of BP were examined to investigate whether CYP3A members are responsible for this reaction. Among the CYP inhibitors used in this study, troleandomycin was the most effective inhibitor of BP dealkylation activity. Diethyldithiocarbamate (a CYP2E1 inhibitor), furafylline (a CYP1A inhibitor) and quinine (a CYP2D inhibitor) had little effect on BP *N*-dealkylation. Similar results were obtained in the immuno-inhibitionstudy. Only anti-rat CYP3A serum inhibited BP *N*-dealkylation (80%), whereas other anti-rat CYP sera were essentially without effects. These results suggest that CYP3A members play a primary role in the *N*-dealkylation from BP to FBPA in rat.

In rat liver microsomes, quinine is approximately 50 times more potent than quinidine as a CYP2D inhibitor (Kobayashi *et al.* 1989). Quinine (2 μM) slightly inhibited BP *N*-dealkylation in this study, whereas the same concentration of quinine inhibited dextromethorphan *O*-demethylation activity, a probe of CYP 2D activity (Küpfer *et al.* 1984, Kerry *et al.* 1993), to 44% of the control. Suzuki *et al.* (1997) reported that thioridazine, which increased the debrisoquine metabolic ratio (Spina *et al.* 1991), co-administration with BP did not affect the plasma concentration of BP. This previous study and the findings of our present study suggest that CYP2D may play a minor role in BP *N*-dealkylation. Some researchers considered the CYP2D6 isoenzyme to be involved in the metabolism of HP in human (Tyndale *et al.* 1991, Llerena *et al.* 1992). Recent studies, however, have reported that both the *N*-dealkylation of HP and the oxidation of RHP back to HP are mainly catalysed by CYP3A4 and not CYP2D6 in human (Fang *et al.* 1997, Pan *et al.*, Kudo and Odomi 1998).

The present authors also investigated whether an inhibitor or an inducer of CYP3A alters BP pharmacokinetics in rat. In previous studies, treatment with 100 mg/kg erythromycin for 7 days or with 80 mg/kg dexamethasone for 2 days affected CYP3A activity (Kolars *et al.* 1992, Murray *et al.* 1992). In our present study, the $t_{1/2}$ of BP was significantly prolonged by treatment of erythromycin, a CYP3A inhibitor and was significantly shortened by treatment of dexamethasone, a CYP3A inducer. Since it was not evaluated which CYP isoform was involved in the *N*-dealkylation of BP at the low concentration observed in the pharmacokinetic studies, it is difficult to know whether CYP3A plays a major role in the intact rat. However, BP metabolism was affected by an inducer and an inhibitor of CYP3A. If other isoforms rather than CYP3A play a main role in BP metabolism, BP pharmacokinetics should be unaffected by an inducer or an inhibitor of CYP3A. BP clearance was reduced by erythromycin and increased by dexamethasone. These results indicate that *in vivo* alteration of CYP3A activity affects BP metabolism and pharmacokinetics, and suggest that the transformation from BP to FBPA, for which CYP3A is responsible, is one of the major steps in the metabolism of BP in rat. In previous clinical studies, carbamazepine, an inducer of CYP3A, significantly decreased plasma concentrations of BP (Otani *et al.* 1997). These previous studies and the findings of the present study suggest that CYP3A contributes more than CYP2D to the metabolism and pharmacokinetics of BP.

In conclusion, it has been shown that CYP3A *N*-dealkylated BP to form FBPA in rat and that alteration of this enzyme activity affected BP pharmacokinetics in rat. Although a simplistic extrapolation of these results to humans is inappropriate, the

results imply that the modification of *in vivo* CYP3A activity affects BP pharmacokinetics. Since a number of drugs and xenobiotics are reported to affect *in vivo* CYP3A activity (Ameer *et al*. 1997, Thummel *et al*. 1998) and since therapeutic and toxic effects of BP are dependent on its plasma concentration, precaution is required in co-administering such a drug or xenobiotic with BP.

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