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Contribution of Cytochrome P450 3A Pathway to Bromocriptine Metabolism and Effects of Ferrous Iron and Hypoxia–re-oxygenation on its Elimination in the Perfused Rat Liver

KEIKO MATSUBAYASHI, HIROSHI MATSUMOTO AND YUKO FUKUI

Department of Legal Medicine, Kyoto University Faculty of Medicine, Kyoto 606-01, Japan

Abstract

The contribution of the cytochrome P450 3A pathway to bromocriptine metabolism, and the effects of ferrous iron and hypoxia–re-oxygenation on its elimination, were evaluated with the perfused rat liver. Outflow profiles of bromocriptine after bolus administration were estimated by moment analysis and dispersion model analysis.

Kinetic parameters were not significantly changed by troleandomycin, a P450 3A inhibitor. The inhibition of bromocriptine metabolism by troleandomycin was $5{\cdot}7 \pm 2{\cdot}4\%$. These findings indicate that cytochrome P450 3A does not play an important role in bromocriptine elimination with the perfused rat liver. Elimination rate constant (k_e) values were significantly increased by ferrous iron perfusion or hypoxia–re-oxygenation. Free-radical generation can, therefore, affect bromocriptine elimination.

Our observations suggest that bromocriptine might be eliminated by scavenging of free radicals in the liver.

Bromocriptine (2-bromo-α-ergocriptine), an ergot alkaloid, is an agonist for dopamine receptors. It is often used for patients with hyperprolactinemia, acromegaly and Parkinson's disease. It is well known that bromocriptine is mainly metabolized in the liver. The hepatic first-pass effect of bromocriptine is large in man and in rats (Schran et al 1980, 1985; Maurer et al 1983). Bromocriptine metabolism by cytochrome P450 has been studied with hepatic microsomes: Moochhala et al (1989) first reported that bromocriptine could interfere with P450-dependent oxidative metabolism of xenobiotics; Ball et al (1992) reported that ergot alkaloids can be metabolized by P450 3A4; and Peyronneau et al (1994) showed that ergopeptides exhibit a particularly high affinity for cytochrome P450 3A and that bromocriptine is hydroxylated by P450 3A at the peptide group. The role of P450 3A4 in the in-vivo metabolism of bromocriptine has not, however, been established.

The metabolism of xenobiotics by cytochrome P450 produces free radicals (White 1991; Coon et al 1992). Yoshikawa et al (1994) and Ogawa (1994) reported that bromocriptine is a free-radical scavenger and a potent antioxidant in rat brain. This finding suggests that bromocriptine might scavenge free radicals in the liver.

We hypothesized that not only hydroxylation by P450 3A but also scavenging of hydroxyl radicals, contributes to hepatic elimination of bromocriptine in-vivo. According to Gores et al (1986), perfusion experiments can be performed independently of the influence of other organ systems, plasma constituents and neural-hormonal effects. Given these considerations, the use of perfused liver might be suitable for establishing the contribution of P450 3A to bromocriptine elimination in whole liver and the role of the scavenging hydroxyl radicals in hepatic elimination. We have investigated the ethanol elimination pathway using perfused rat liver (Matsumoto et al 1994, 1996). The roles of alcohol dehydrogenase and P450 2E1 in hepatic ethanol elimination were estimated by statistical moment analysis and dispersion model analysis under perfusion of their inhibitors (Matsumoto et al 1996).

In this study to prove the hypothesis described above we have used perfused rat liver to estimate the contribution of the P450 3A pathway to bromocriptine elimination. The effects of troleandomycin (a P450 3A inhibitor), aminotriazole (3-amino-1,2,4-triazole), ferrous iron (Fe^{2+}) and hypoxia–re-oxygenation have been studied by pharmacokinetic analysis.

Correspondence: K. Matsubayashi, Department of Legal Medicine, Kyoto University Faculty of Medicine, Sakyo-ku, Kyoto 606-01, Japan.

Materials and Methods

Chemicals

Bromocriptine was obtained from Sando Pharmaceutical Co., (Kyoto, Japan). Other chemicals were purchased from Nacalai Tesque Inc. (Kyoto, Japan).

Animals

Male Wistar rats, 230–250 g, were freely provided with laboratory water and chow. Animals were fasted overnight before the experiments.

Liver preparation

The single-pass perfused rat liver was prepared in-situ as described previously (Matsumoto et al 1994, 1996; Evans et al 1991). Rats were anaesthetized with pentobarbital sodium (50 mg kg^{-1}, i.p.). The bile duct was cannulated with a PE-10 tube (0·28 mm i.d.; Clay Adams, NJ), the portal vein with a PE-160 tube (1·14 mm i.d.) as an inflow catheter and the vena cava inferior through the right atrium with a PE-240 tube (1·67 mm i.d.) as an outflow catheter. The livers were perfused

at a constant flow rate of 14·4 mL min^{-1} with Krebs-Henseleit bicarbonate buffer (pH 7·4 at 37°C) containing 11·1 mM glucose and oxygenated with 95% O_2–5% CO_2 at 100 mL min^{-1} during its passage through the Hamilton lung. The red blood cells were not included in the perfusate to avoid their interaction with drug disposition in the liver (Yasui et al 1995).

Bromocriptine administration and perfusate sampling

Bromocriptine (30 mg) was dissolved in 30% (v/v) methanol (100 mL). Bromocriptine dose was delivered into a sample injector (100 μL) (Eyela SV-6010; Tokyo Rikakikai, Tokyo, Japan) and rapidly injected into the inflow catheter. The methanol concentration was high but the amount was small. To estimate the effect of dose on bromocriptine elimination we used three doses of 15, 20 and 30 μg, which are used for clinical treatment of man. For the inhibition and hypoxia–re-oxygenation experiments, a fixed dose of 30 μg was used. The outflow perfusate was collected into pre-weighed vials at 3-s intervals for 1 min after bolus-input of bromocriptine. Bile was collected at 5-min intervals during the experiment. Liver viability was assessed by monitoring portal pressure (Hijioka et al 1991), perfusate recovery, bile flow and macroscopic appearance (Gores et al 1986; Evans et al 1991). PO_2, PCO_2, and pH in the inflow and outflow perfusates were monitored throughout. After the experiment, perfusion was evaluated by the coloration of Evans Blue perfused in the liver, and the liver was examined microscopically.

Experimental protocol

Five perfusion experiments were performed (Fig. 1): effect of bromocriptine doses on kinetic parameters; perfusion of a P450 3A inhibitor, troleandomycin; perfusion of a catalase inhibitor, aminotriazole; ferrous iron perfusion; and hypoxia–re-oxygenation perfusion.

In the first experiment, after liver perfusion for 15 min, a bolus bromocriptine dose of 15, 20 or 30 μg was injected into the inflow catheter. In the other experiments, a 30-μg dose was administered. In the second and third experiments troleandomycin (60 μM, Berthou et al 1994; Chang et al 1994) or aminotriazole (20 μM, Williams et al 1985) was added to the perfusate (Fig. 1a). In the fourth experiment ferrous iron solutions (15 μM $FeSO_4$) were prepared using perfusate that had been rendered anaerobic by vigorous bubbling with 100% N_2 for at least 30 min before addition of ferrous sulfate as described previously (Brissot et al 1985; Wright et al 1986). Ferrous iron perfusion was begun after perfusion of troleandomycin for 15 min (Fig. 1b). In the fifth experiment, hypoxia was achieved by removing the Hamilton lung and the perfusate was re-oxygenated by use of the Hamilton lung (Fig. 1c).

HPLC analysis of bromocriptine

A Shimadzu (Kyoto, Japan) HPLC (LC-6A pump), a model SPD-6AV UV detector set at 254 nm and a SIL-9A injector were used for the determination of bromocriptine concentration in the outflow perfusate. The chromatograph was equipped with a 6 cm × 150 mm i.d., 5 μm particle size, Cosmosil (Nacalai Tesque, Kyoto, Japan) C_{18}-MS column; the mobile phase was 65 : 35 (v/v) methanol–water that was 0·01 M in 1-heptanesulphonic acid (Larsen et al 1979); the flow rate was 1·0 mL min^{-1}. The retention time of bromocriptine was 12 min. The detectable level of bromocriptine concentration was 10 ng mL^{-1} and above.

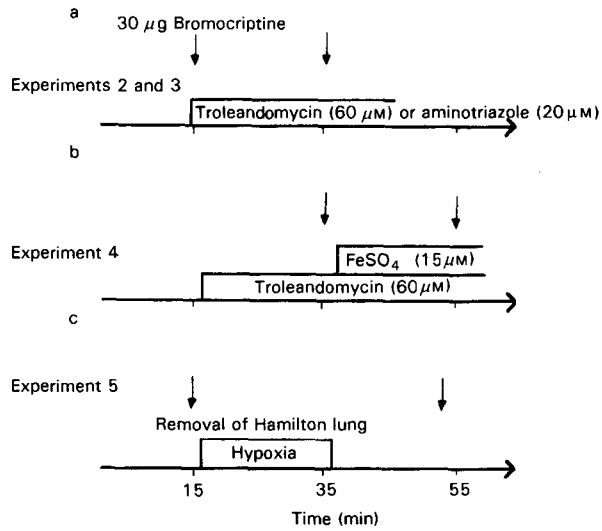

Fig. 1. Experimental protocols illustrating administration of a bolus bromocriptine dose (30 μg; arrows) to perfused rat liver. a, Second and third experiments; after perfusion of Krebs-Henseleit buffer for 15 min as a control, inhibitor (60 μM troleandomycin or 20 μM aminotriazole) was added to the perfusate for 20 min. b, Fourth experiment; after perfusion of 60 μM troleandomycin for 20 min, $FeSO_4$ (15 μM) was perfused for 20 min. c, Fifth experiment; after perfusion of Krebs-Henseleit buffer for 15 min as a control, the perfusate was made hypoxic by removal of the Hamilton lung for 20 min. After hypoxia, the perfusate was re-oxygenated for 15 min by use of the Hamilton lung.

Data analysis

Sampling times were corrected by use of sampling weights. All bromocriptine outflow profiles were estimated by statistical moment analysis (Yamaoka et al 1978b; Yano et al 1989) and the two-compartment dispersion model (Yano et al 1989, 1990). Moments, the area under the curves (AUC, μg s mL^{-1}), the mean transit times (MTT, s) and the variance of transit time (VTT, s^2), were obtained by the linear trapezoidal rule for outflow bromocriptine concentration–time courses. The recovery ratio (F, %) and the hepatic volume of distribution (V_H, mL) were calculated using the equations:

$$F = 100 \times AUC \times Q/D \quad (1)$$

and

$$V_H = Q \times MTT \quad (2)$$

where Q (mL min^{-1}) is the flow rate of perfusate and D (μg) is the injected dose. First pass effect (%) was estimated as 100-F.

A Laplace-transformed equation of the two-dispersion model was fitted to the outflow concentration–time courses using the non-linear least-squares method program, Multi (FILT, fast inverse Laplace transform) (Yano et al 1989). The weighting function of $1/[\tilde{C}(S)]^2$ was used for non-linear

regression. Equation 3 is the Laplace transformed solution of the outflow profile after the impulse input of a drug (Yano et al 1990):

$$\tilde{C}(s) = \frac{D}{Q} \cdot \exp\left[\left\{\frac{Q}{2D_c} - \sqrt{\left(\frac{Q}{2D_c}\right)^2 + \frac{1}{D_c}\left(s + k_{12} + k_e - \frac{k_{12} \cdot k_{21}}{s + k_{21}}\right)}\right\} V_B\right] \quad (3)$$

where D_c ($mL^2\ min^{-1}$) is the corrected dispersion coefficient, k_e (min^{-1}) is the first-order elimination rate constant from the perfusate mainly into the hepatic tissue, k_{12} and k_{21} (min^{-1}) are the forward and backward partition rate constants between the perfusate and tissues, and V_B (mL) is the volume of hepatic blood space which represents the combined volumes of the sinusoid and the space of Disse. The partition ratio (k′) is defined by k_{12}/k_{21}. The efficiency number, R_N, (Roberts & Rowland 1986) is given by:

$$R_N = k_e \cdot V_B/Q \quad (4)$$

The contribution of pathways inhibited by perfusion of drugs was calculated by use of the efficiency number. The inhibition ratio ($R_{INHIBIT}$) was estimated with the equation (Matsumoto et al 1996):

$$R_{INHIBIT} = (R_{N\ CONTROL} - R_{N\ INHIBIT})/R_{N\ CONTROL} \quad (5)$$

Statistical analysis

All results were expressed as means ± s.e.m. Student's *t*-test after the *F* test for variance, and one-way analysis of variance with Duncan's multiple range test were used for comparisons of two or more groups, respectively. A probability level of $P < 0.05$ was considered as indicative of significance.

Results

The mean weight of rats was 241 ± 3 g and the wet liver weight was 7·4 ± 0·3 g. The flow rate of 14·4 mL min^{-1} was 1·95 ± 0·07 mL min^{-1} g^{-1} liver weight. There was no change of bile production in any of the experiments. The mean value of bile flow was 0·93 ± 0·07 μL min^{-1} g^{-1} liver, which was close to the standard value reported by Gores et al (1986). Portal pressure was 5·3 ± 0·1 cm H_2O, which was similar to previously reported values (Hijioka et al 1991). The LDH value in the outflow perfusate was 1·4 ± 0·4 int. units L^{-1} except during hypoxia–re-oxygenation (3·8 ± 0·6 int. units L^{-1}) (Fig. 2). No pathological changes, e.g. necrosis, were found microscopically in any liver. Liver viability in the this study was, therefore, confirmed.

For bromocriptine doses of 15, 20 and 30 μg, MTT, VTT and V_H values were not significantly different. The recovery ratio values were not significantly different between doses of 15 and 20 μg, but were significantly higher for the 30-μg dose.

In perfusion with troleandomycin (the troleandomycin group) and troleandomycin and ferrous iron (the Fe^{2+} group), a similar one-peak pattern was obtained for all outflow (Fig. 3). The slope and peak height of courses in the Fe^{2+} group were gentler and lower than those in the troleandomycin group. The bromocriptine peak concentrations in the Fe^{2+} group were one-thirtieth of those in the troleandomycin group. Under ferrous iron perfusion, bromocriptine was undetectable 15 s after administration (Fig. 3). Fig. 4 shows the effect of hypoxia–re-oxygenation on bromocriptine concentration–time curves. In hypoxia–re-oxygenation, the properties of outflow curve was similar to those from ferrous iron perfusion, and the bromocriptine peak concentrations were one-sixth of those from the control experiment (Fig. 4).

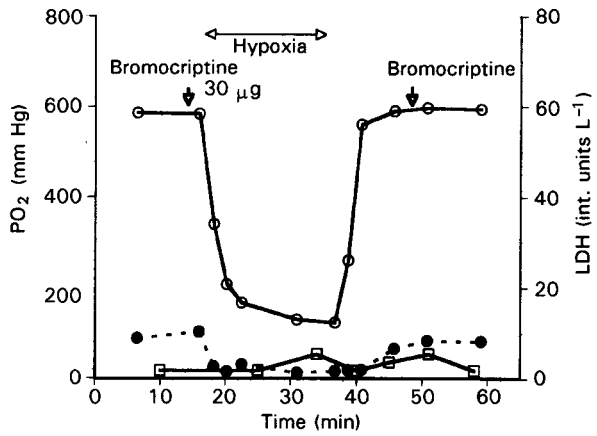

FIG. 2. Oxygen pressures in inflow (○) and outflow (●), and LDH activity (□) in outflow during hypoxia–re-oxygenation. A bolus bromocriptine dose (30 μg) was added to the inflow during oxygenation (control). The hypoxic state was achieved for 20 min by the removal of the Hamilton lung. The perfusate was then re-oxygenated for 15 min and a second 30-μg dose was administered.

The recovery ratio value in the control was 9·5 ± 1·5 (%) (Table 1). The hepatic first-pass effect of bromocriptine was 90·5 ± 1·5 (%). Moments in the troleandomycin and aminotriazole groups were not significantly different from that in the control group (Table 1). The presence of ferrous iron in the perfusate resulted in a reduction in all the moments (Table 1). The recovery ratio value in particular was reduced to a value close to zero ($P < 0.005$). The AUC and recovery ratio values were significantly reduced by hypoxia–re-oxygenation ($P < 0.01$ and $P < 0.005$, respectively) (Table 1).

The one- and two-compartment models were compared according to the Akaike information criterion (Yamaoka et al 1978a), with minimum values regarded as the best representation of the concentration–time course data. We obtained smaller Akaike information criterion values for the two-compartment dispersion model than the one-compartment models. The two-compartment dispersion model was suitable for describing the bromocriptine outflow profiles in the perfused liver (Figs 3 and 4). The pharmacokinetic parameters obtained by fitting the two-compartment dispersion model to these profiles are shown in Table 2. In the troleandomycin and aminotriazole groups there were no significant differences among the parameters. The inhibition rate of troleandomycin and aminotriazole calculated from equation 5 were 5·7 ± 2·4 and 11·0 ± 0·9 (%), respectively. The pathway via cytochrome P450 3A inhibited by troleandomycin was, therefore, responsible for 5·7 ± 2·4% of hepatic bromocriptine elimination. Under ferrous iron perfusion, only the k_e value was significantly increased ($P < 0.005$) (Table 2). Similarly, in hypoxia–re-oxygenation, the k_e value was significantly increased ($P < 0.05$) (Table 2). No changes in D_c and V_B were observed. In ferrous iron perfusion the hepatic volume of

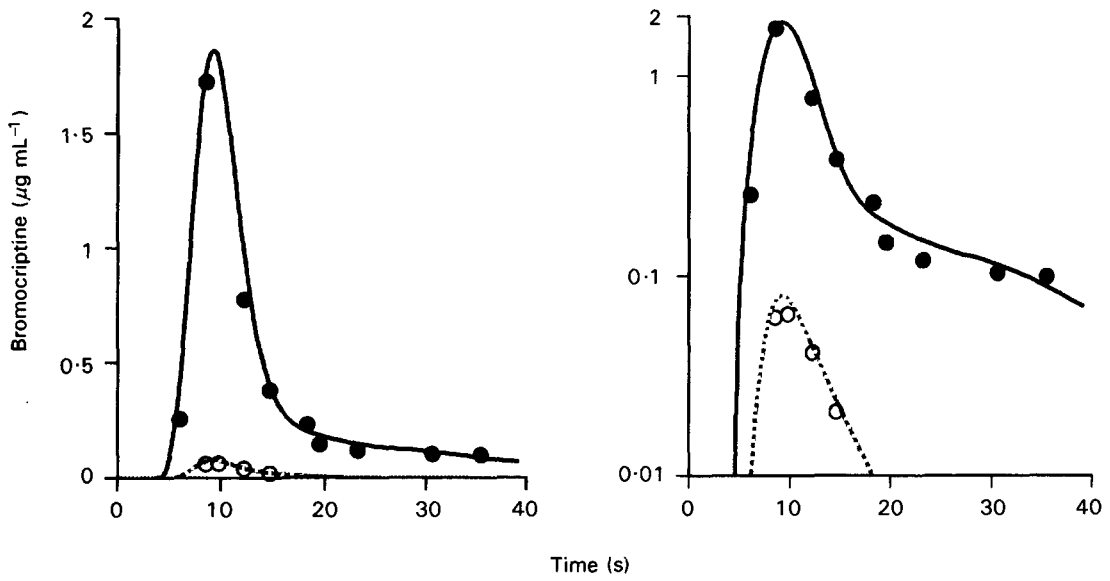

FIG. 3. Effect of troleandomycin and troleandomycin-ferrous iron perfusion on bromocriptine concentration–time courses of a typical experiment. (●) Troleandmycin, (○) troleandmycin-ferrous iron. The solid and dotted lines represent fitting curves for the two-compartment dispersion model. Left, linear plots; right, semi-logarithmic plots.

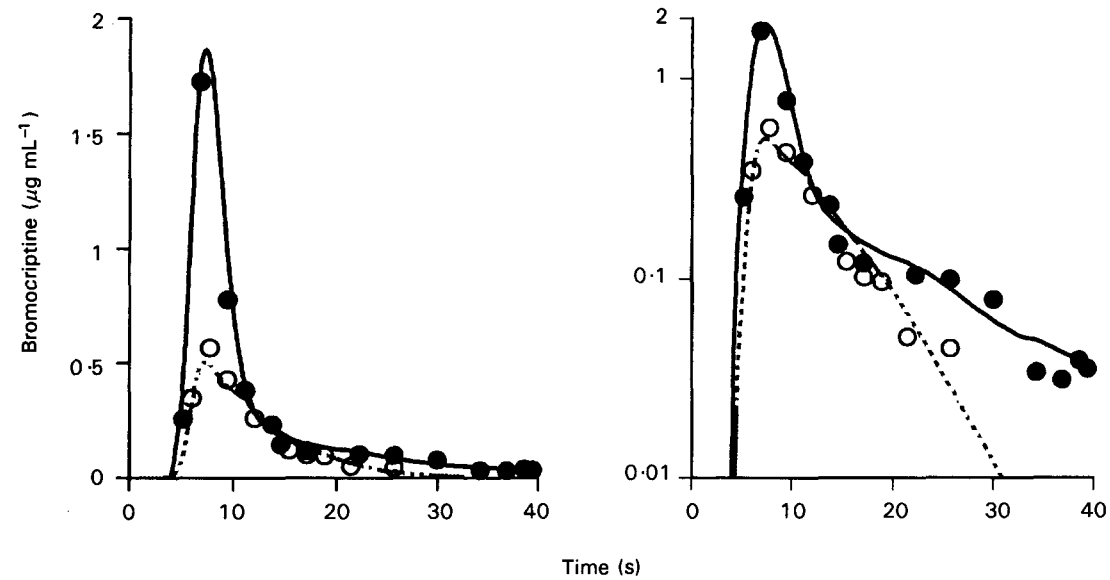

FIG. 4. Typical effect of hypoxia–re-oxygenation on bromocriptine concentration–time courses: (●) control, (○) hypoxia–re-oxygenation. The solid and dotted lines represent fitting curves for the two-compartment dispersion model. Left, linear plots; right, semi-logarithmic plots.

distribution (V_H), calculated from $V_B(1+k')$, and the tissue volume of distribution, calculated from $V_H - V_B$, were $3{\cdot}0 \pm 0{\cdot}2$ and $0{\cdot}38 \pm 0{\cdot}1$ mL, respectively; in hypoxia–re-oxygenation they were $3{\cdot}2 \pm 0{\cdot}3$ and $0{\cdot}65 \pm 0{\cdot}2$ mL, respectively. No increase in hepatic distribution of bromocriptine was observed under these conditions. These findings indicated that the uptake of bromocriptine into the perfused rat liver was raised by ferrous perfusion and hypoxia–re-oxygenation.

Discussion

On the basis of the amount of orally administered radioactivity monitored and the AUC ratios observed in rats after oral and intravenous doses a high hepatic first-pass effect has been reported for bromocriptine (Maurer et al 1983; Schran et al 1985). We calculated a hepatic first-pass effect of 90·5% using the recovery ratio. This value is similar to those reported by Schran et al and Maurer et al and proves that bromocriptine is mainly metabolized in the liver.

By use of troleandomycin, a selective inhibitor of the cytochrome P450 3A subfamily (Berthou et al 1994; Chang et al 1994), we estimated the contribution of the cytochrome P450 3A pathway to bromocriptine metabolism. The pharmacokinetic parameters in the troleandomycin groups were not significantly different from those in the control group. On the basis of previous reports that bromocriptine can be metabolized by P450 3A in microsomes (Ball et al 1992; Peyronneau et al 1994), we hypothesized that troleandomycin might inhibit

Table 1. Effect of troleandomycin, aminotriazole, troleandomycin and ferrous iron (Fe^{2+}) and hypoxia–re-oxygenation on pharmacokinetic parameters obtained by moment analysis of bromocriptine outflow profiles from perfused rat liver.

	n	Area under the curve ($\mu g\ s\ mL^{-1}$)	Mean transit time (s)	Variance of transit time (s^2)	Hepatic volume of distribution (mL)	Recovery ratio (%)
Control	10	12·8 ± 0·5	11·0 ± 0·3	40·8 ± 7·8	2·6 ± 0·2	9·5 ± 1·5
Troleandomycin	10	13·8 ± 1·0	12·6 ± 0·8	44·2 ± 4·1	2·9 ± 0·3	10·2 ± 0·8
Control	8	11·5 ± 0·5	11·8 ± 0·6	42·2 ± 9·4	2·8 ± 0·1	8·0 ± 0·3
Aminotriazole	8	12·3 ± 1·1	12·8 ± 0·9	42·0 ± 8·2	3·1 ± 0·1	5·5 ± 0·6
Troleandomycin	5	14·9 ± 2·2	11·5 ± 0·5	46·6 ± 9·4	2·8 ± 0·1	10·5 ± 1·7
Troleandomycin–Fe^{2+}	5	0·94 ± 0·14§	7·0 ± 1·3†	24·8 ± 3·6†	1·7 ± 0·3†	0·8 ± 0·1§
Control	6	13·8 ± 1·3	12·3 ± 0·5	46·3 ± 8·3	3·0 ± 0·1	11·0 ± 1·0
Hypoxia–re-oxygenation	6	5·9 ± 1·5*	12·3 ± 0·5	40·9 ± 7·0	3·0 ± 0·1	4·7 ± 1·2‡

Values are means ± s.e.m. *$P < 0.01$, ‡$P < 0.005$ compared with control; † $P < 0.01$, §$P < 0.005$ compared with tolerendomycin.

Table 2. Effect of troleandomycin, aminotriazole, troleandomycin and ferrous iron (Fe^{2+}) and hypoxia–re-oxygenation on pharmacokinetic parameters obtained by fitting two-compartment dispersion model to bromocriptine outflow profiles from the perfused rat liver.

	Corrected dispersion coefficient ($mL^2\ min^{-1}$)	Volume of hepatic blood space (mL)	Forward partition rate constant between perfusate and tissues (min^{-1})	Backward partition rate constant between perfusate and tissues (min^{-1})	Partition ratio	Elimination rate constant (min^{-1})
Control	0·35 ± 0·1	2·2 ± 0·2	6·6 ± 2·6	16·4 ± 2·9	0·37 ± 0·10	17·9 ± 0·8
Troleandomycin	0·35 ± 0·04	1·9 ± 0·1	9·2 ± 2·3	19·3 ± 3·7	0·46 ± 0·06	17·9 ± 1·3
Control	0·60 ± 0·1	2·4 ± 0·1	3·4 ± 0·7	12·0 ± 1·7	0·35 ± 0·03	19·9 ± 2·0
Aminotriazole	0·66 ± 0·2	2·4 ± 0·1	3·7 ± 0·7	7·8 ± 0·8	0·46 ± 0·05	17·8 ± 2·6
Troleandomycin	0·34 ± 0·2	2·1 ± 0·3	10·5 ± 3·2	16·8 ± 0·4	0·62 ± 0·2	17·9 ± 2·3
Troleandomycin–Fe^{2+}	0·76 ± 0·9	2·9 ± 0·2	8·4 ± 2·3	16·5 ± 8·7	1·47 ± 0·9	41·0 ± 5·6†
Control	0·63 ± 0·3	2·2 ± 0·2	4·9 ± 1·0	15·6 ± 3·7	0·38 ± 0·1	16·5 ± 1·8
Hypoxia–re-oxygenation	0·71 ± 0·2	2·6 ± 0·2	6·6 ± 2·4	12·6 ± 2·5	0·51 ± 0·1	39·2 ± 8·6*

Values are means s.e.m. (n = 5) *$P < 0.05$, significant compared with control; † $P < 0.005$, significant compared with troleandomycin.

bromocriptine metabolism via P450 3A in the liver. The inhibition of bromocriptine metabolism in the troleandomycin group of experiments was, however, found to be 5·7%, lower than we expected. It is found that cytochrome P450 3A is a minor contributor to elimination of bromocriptine in the liver. Because other fractions and organelles (as well as the microsome) can affect bromocriptine metabolism, we evaluated other bromocriptine elimination pathways in the perfused liver.

On the basis of reports that bromocriptine is a scavenger of hydroxyl radicals (Ogawa 1994; Yoshikawa et al 1994), we evaluated the effects of aminotriazole, ferrous iron and hypoxia–re-oxygenation on hepatic bromocriptine elimination. An increase in H_2O_2 by a catalase inhibitor, e.g. aminotriazole, results in an increase in hydroxyl radicals. The kinetic parameters of bromocriptine were not, however, significantly changed by perfusion of aminotriazole and do hydroxyl radicals in the liver might not be markedly produced under perfusion of aminotriazole, or the pathway via catalase might not play a role in bromocriptine metabolism. Iron is known to accelerate the Haber-Weiss reaction and the formation of hydroxyl radicals (Braughler et al 1986). The combination of H_2O_2 and ferrous iron, known as Fenton's reagent, has been used to generate hydroxyl radicals (Braughler et al 1986; Yoshikawa et al 1994). Bucher et al (1983) reported that ferrous iron alone will also initiate lipid peroxidation and so the cytochrome P450s pathway and radical generation can both be activated by ferrous iron perfusion. To avoid activating cytochrome P450 3A by ferrous iron perfusion, we maintained permanent perfusion of troleandomycin during the experiment. The decrease in AUC and recovery ratio and the increase in K_e under iron perfusion suggest that bromocriptine elimination is increased by generation of radicals and activation of P450s other than the 3A subfamily. Under ferrous iron perfusion, V_H measured by moment analysis was reduced, but V_H obtained by dispersion model analysis was unchanged; this difference was attributed to the difficulty in estimating the terminal phase of time curves. The terminal phase of the curve was not exactly estimated by moment analysis because of undetected low concentrations. On the other hand, the terminal phase of the curve was rigorously estimated by good fitting of the two-compartment dispersion model and the values of V_H obtained should be similar to the real value.

Several studies have shown that oxygen free-radicals are implicated in reperfusion–re-oxygenation damage in various

organs (Bysani et al 1990; Younes et al 1992; Paller & Jacob 1994). Generally, hypoxia–re-oxygenation seems to reduce metabolism and lead to higher values for AUC and recovery ratio. The metabolic pathways through cytochrome P450 3A should be inactivated by hypoxia–re-oxygenation. In our hypoxia–re-oxygenation experiment, however, decreases in AUC and recovery ratio were found by moment analysis and an increase in K_e was found by use of the dispersion model. These findings indicate that bromocriptine elimination was increased and suggests that other elimination pathways are activated by our hypoxia–re-oxygenation method. Hypoxia used in the present study was not anoxia (100% N_2 or 95% N_2–5% CO_2) but was achieved by removing the Hamilton lung and perfusing Krebs-Henseleit bicarbonate buffer equilibrated with air for 20 min. We preserved liver viability to enable estimation of bromocriptine elimination. No changes in D_c and V_B were observed. D_c is a characteristic of the organic anatomical properties (Chou et al 1993). Yasui et al (1995) described an increase in V_B as a result of liver damage. None of the experiments showed anatomical or pathological changes in the liver. In the free radical-generating system, 95–100% N_2 gas has been generally used to produce an anoxic state for 1–4 h (Littauer & Groot 1992; Younes et al 1992; Caraceni et al 1994). These conditions affect hepatic disposition; an increase in LDH release and reduced bile flow have been shown by Younes et al (1992) and Caraceni et al (1994). In fact, we found LDH release by hypoxia–re-oxygenation to be much lower in the present study than in other reports on hypoxia–re-oxygenation. Fewer free radicals might, therefore, have been generated in this study than in the radical-generating experiments described previously. Our results under hypoxia–re-oxygenation support the hypothesis that bromocriptine elimination is increased by generation of free radicals. In this study the kinetic parameters obtained from the dispersion model under ferrous iron perfusion were similar to those under hypoxia–re-oxygenation. This finding also indicated that bromocriptine elimination was increased by the same mechanism of free-radical generation. Yoshikawa et al (1994) and Ogawa (1994) reported that bromocriptine is a free-radical scavenger and antioxidant. Bromocriptine might be eliminated by scavenging of free radicals in the liver.

In conclusion, the cytochrome P450 3A pathway makes a small contribution to hepatic bromocriptine elimination. The bromocriptine elimination rate was increased by ferrous iron perfusion and hypoxia–re-oxygenation, which suggests that free radical generation can affect bromocriptine elimination.

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