J. Pharm. Pharmacol. 1996, 48: 1185–1189
Received January 29, 1996
Accepted May 9, 1996

In-vitro Metabolic Interaction of Bunitrolol Enantiomers in Rabbit Liver Microsomes

SHIZUO NARIMATSU, TAKAO MIZUKAMI, YONG HUANG, YASUHIRO MASUBUCHI AND TOKUJI SUZUKI

Laboratory of Biopharmaceutics, Faculty of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263, Japan

Abstract

We have examined the 4-hydroxylation of bunitrolol in rabbit and rat liver microsomes.

Significant species differences (rabbit < rat of both sexes) and sex (male > female of both species) were observed in the formation of 4-hydroxybunitrolol from racemic bunitrolol (10 μM). The 4-hydroxylation of bunitrolol racemate and enantiomers showed biphasic kinetics, a low-K_m system and a high-K_m system, in liver microsomes from rabbits of both sexes. There were significant differences in K_m and V_{max} values [(+) > (−)] for 4-hydroxylations of (+)-bunitrolol and (−)-bunitrolol in the low-K_m system. Furthermore, the rate of clearance (V_{max}/K_m) was 20- to 200-fold for the low-K_m system compared with the high-K_m system, indicating that enzymes in the low-K_m system play a major part in the rabbit liver microsomal bunitrolol metabolism. Inhibition studies using cytochrome P450 inhibitors such as quinidine, quinine, and α-naphthoflavone or polyclonal antibodies raised against rat P450-2D and -1A enzymes did not make clear which P450 enzymes are involved in bunitrolol 4-hydroxylation in rabbit liver microsomes. The 4-hydroxylase activity of (+)-bunitrolol was slightly higher than that of (−)-bunitrolol in separated incubations containing male rabbit liver microsomes and an enantiomer concentration of 10 μM. However, the 4-hydroxylation of (+)-bunitrolol (10 μM) was markedly suppressed in the presence of its antipode (10 μM), whereas (−)-bunitrolol 4-hydroxylation was not affected by the presence of its antipode, resulting in a change of the stereoselectivity from (+) > (−) for enantiomer to (+) < (−) for racemate.

The difference in the Michaelis constants in the low-K_m system, where the K_m value of (−)-bunitrolol is one-eighth that of (+)-bunitrolol, is thought to cause the change in the stereoselectivity in rabbit liver microsome-mediated bunitrolol 4-hydroxylation.

Bunitrolol has an asymmetric carbon on a side chain, and is administered as a racemic mixture of levo-(−)-bunitrolol and dextro-(+)-bunitrolol, the former being about 20 times more potent than the latter as a β-blocker. The major metabolic pathway of bunitrolol is 4-hydroxylation of the phenyl ring in mice and rats (Suzuki & Rikihisa 1979) and man (Kono et al 1987). We recently reported that cytochrome P450 (P450) enzyme belonging to the P450-2D subfamily was involved in bunitrolol 4-hydroxylation in liver microsomes from rats (Suzuki et al 1991) and man (Narimatsu et al 1994b). More recently we have developed a high-performance liquid chromatography (HPLC) method for assay of enantiomeric 4-hydroxybunitrolol formed from racemic bunitrolol, and demonstrated an enantiomer/enantiomer interaction in the oxidative metabolism of bunitrolol in rat liver microsomes and P450-2D enzymes, commonly involved in the 4-hydroxylation of both enantiomers in rats (Narimatsu et al 1994a).

When bunitrolol enantiomers were intravenously administered to adult male rabbits, venous blood levels of (−)-bunitrolol were significantly higher than those of (+)-bunitrolol (unpublished data from the Nippon Boehringer Ingelheim Co.). To understand the interaction in the rabbit between bunitrolol and P450 as a typical hepatic drug-metabolizing enzyme, we have kinetically analysed the oxidative metabolism of bunitrolol racemate and enantiomers in liver microsomes from adult rabbits.

Correspondence: S. Narimatsu, Laboratory of Biopharmaceutics, Faculty of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263, Japan.

Materials and Methods

Drugs

Racemic and enantiomeric bunitrolol and racemic 4-hydroxybunitrolol as hydrochlorides were kindly supplied by the Nippon Boehringer Ingelheim Co. (Hyogo, Japan); debrisoquine hemisulphate was from the Hoffman La-Roche Co. (Basel, Switzerland). Quinine sulphate was purchased from Fujisawa Pharmaceutical Ind. (Osaka, Japan); quinidine hydrochloride was from the Sigma Chemical Co. (St Louis, MO, USA); α-naphthoflavone was from Wako Pure Chemical Ind. (Osaka, Japan). Other chemicals and solvents used were of analytical grade.

Animals

Adult Japanese white rabbits of both sexes (3 months old) and adult Wistar rats of both sexes (7 weeks old) were obtained from Takasugi Experimental Animals (Kasukabe, Japan). The animals were allowed free access to food and water for at least 1 week prior to use. After 12 h of starvation, the animals were killed by sanguination, and liver microsomes were prepared by the method of Omura & Sato (1964).

Assay

4-Hydroxybunitrolol enantiomers were determined as acetyl derivatives by HPLC (Narimatsu et al 1994a). Racemic 4-hydroxybunitrolol was determined without derivatization using reversed-phase HPLC (Narimatsu et al 1994b). Briefly, a 1-mL incubation mixture contained 10 mM glucose-6-phosphate (G-

6-P), 2 U G-6-P dehydrogenase, 0·5 mM NADPH, 8 mM $MgCl_2$, 1 mg mL^{-1} microsomal protein, 10 μM bunitrolol and 154 mM potassium phosphate buffer (pH 7.4) to make a final volume of 1·0 mL. After 5 min of preincubation under air at 37°C, reaction was started by adding the NADPH and was stopped 1 min later by adding 0·5 mL of 1 M NaOH.

When 4-hydroxybunitrolol enantiomers were assayed, 1 mL of 1 M carbonate buffer (pH 9·6) and imipramine (3·6 nmol) as an internal standard were added to the medium, and metabolites were extracted into ethyl acetate by shaking. 4-Hydroxybunitrolol enantiomers were then acetylated with acetic anhydride (50 μL) in pyridine (100 μL) followed by heating at 60°C for 2 h. The solvent and reagent were evaporated and the residue was dissolved in 100 μL of an HPLC mobile phase (*n*-hexane-ethanol-diethylamine = 90:25:0.1, by volume). When 4-hydroxybunitrolol racemate was assayed, 1 mL of 1 M carbonate buffer (pH 9·6) and α-naphthylethylenediamine (50 ng) were added to the medium, and metabolites were extracted into ethyl acetate. The solvent was evaporated, and the residue was dissolved in 100 μL of an HPLC mobile phase (methanol-acetonitrile-water-acetic acid = 22:18:60:1, by volume). In kinetic studies, racemic enantiomeric bunitrolol (0·5 to 1000 μM) was employed.

Inhibition studies

Various inhibitors or substrate of P450 (quinidine, quinine, debrisoquine and α-naphthoflavone) were added at various concentrations to the reaction medium containing bunitrolol enantiomers (30 μM or 1 mM). In immunochemical studies, polyclonal antibodies (immunoglobulin G fraction) raised against rat P450-2D (Suzuki et al 1992) and -1A enzymes (Fujita et al 1988) were preincubated with rabbit liver microsomes at 25°C for 30 min, and then bunitrolol 4-hydroxylase activities were assayed as described above.

HPLC conditions

The HPLC apparatus consisted of a Hitachi 655 liquid chromatograph equipped with a 650-10S fluorescence detector (excitation/emission wavelength, 310/380 nm). In the assay of racemic 4-hydroxybunitrolol: column, Inertsil ODS (4·6 mm i.d. × 25 cm, GL Science Ltd., Tokyo, Japan); flow rate 1·2 mL min^{-1}. In the assay of 4-hydroxybunitrolol enantiomers: column, Optipak XC (4·6 mm i.d. × 30 cm, Waters Co., Tokyo, Japan); flow rate 1 mL min^{-1}.

Others

Protein concentration was determined by the method of Lowry et al (1951) using bovine serum albumin as standard. Enzyme kinetic parameters (K_m, V_{max} and K_i) were analysed according to a non-linear least-square regression analysis based on a simplex method (Yamaoka et al 1981). Statistical significance was calculated by Student's *t*-test and paired *t*-test.

Results

Oxidative metabolism of bunitrolol racemate

Using a substrate concentration of 10 μM, oxidative metabolism of racemic bunitrolol was examined in liver microsomes from male rabbits. 4-Hydroxybunitrolol was the only bunitrolol metabolite detected under the conditions used. The bunitrolol 4-hydroxylase activities in liver microsomes from rabbits of both sexes (male: 0·59 ± 0·03, female 0·31 ± 0·06) were significantly lower than those from rats of both sexes

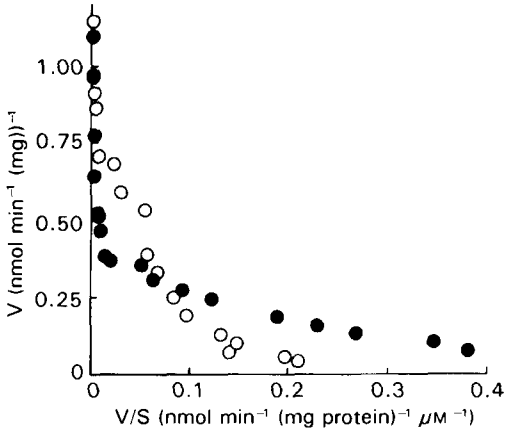

FIG. 1. Typical Eadie-Hofstee plots showing 4-hydroxylations of bunitrolol enantiomers in rabbit liver microsomes. Bunitrolol enantiomers (0.5 to 1000 μM) were incubated with liver microsomes from male rabbits in the presence of an NADPH-generating system, and 4-hydroxybunitrolol enantiomers formed were determined by the HPLC under the conditions described in Materials and Methods. Each point represents the mean value of duplicate determinations. ○ (+)-bunitrolol; ● (−)-bunitrolol.

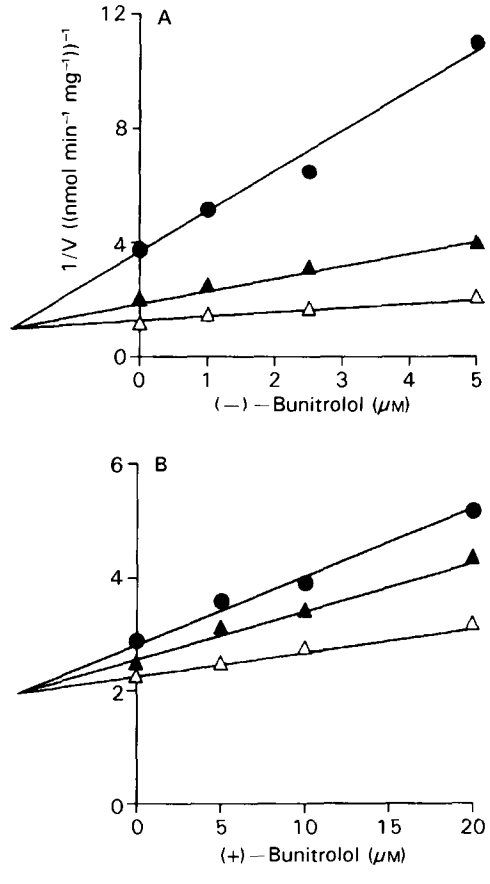

FIG. 2. Dixon plots showing competitive inhibition between bunitrolol enantiomers in rabbit liver microsomes. A, (−)-bunitrolol (0, 1, 2.5, and 5 μM) was added as inhibitor to the incubation medium containing (+)-bunitrolol (5, 20, and 50 μM) as substrate; B, (+)-bunitrolol (0, 5, 10, and 20 μM) was added as inhibitor to the incubation medium containing (−)-bunitrolol (2, 4, and 8 μM) as substrate. Each point represents the mean value of duplicated determinations. The figure shows typical results of three determinations.

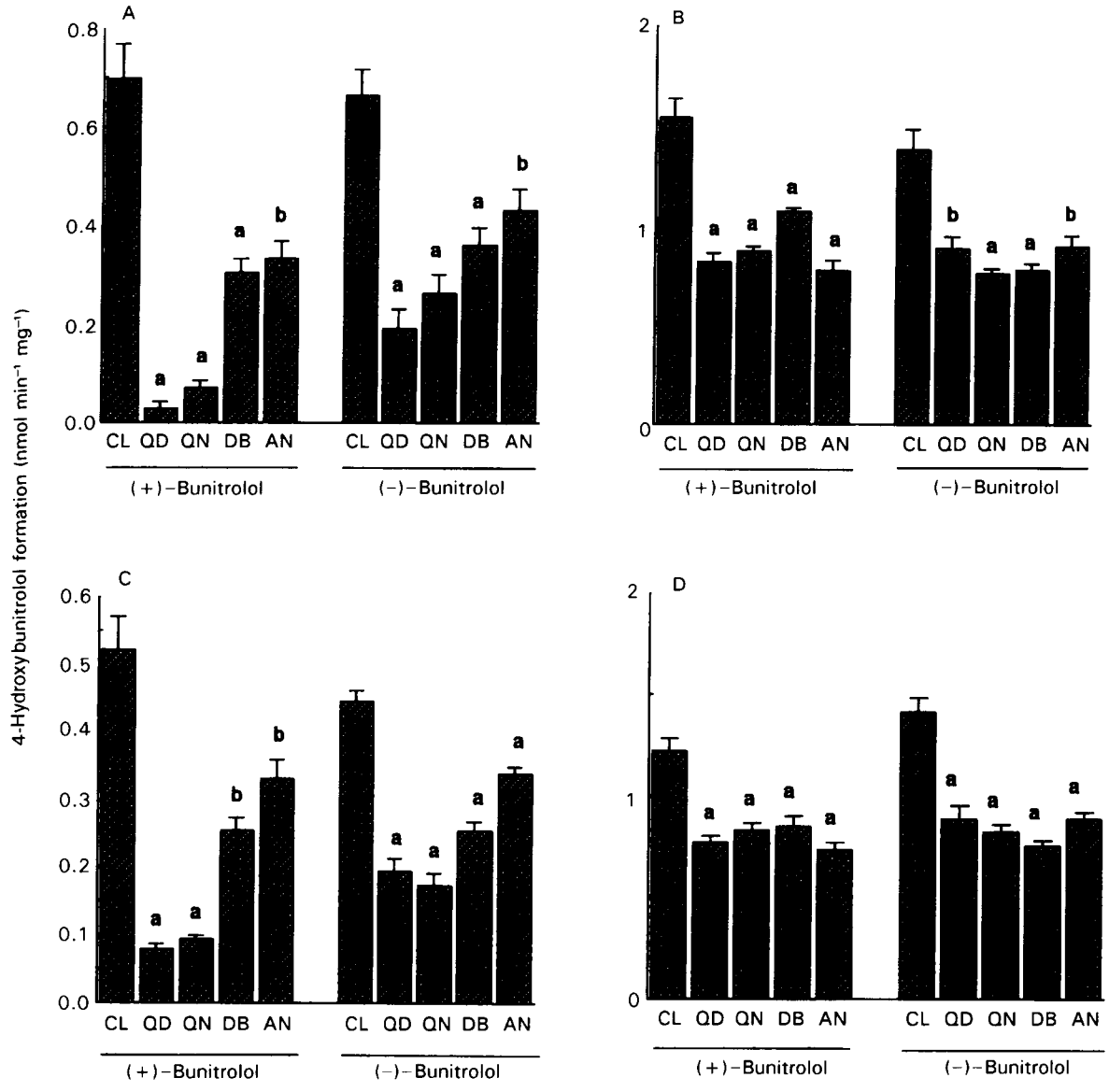

FIG. 3. Effects of various inhibitors on the metabolism of bunitrolol enantiomers in rabbit liver microsomes. Bunitrolol enantiomers [30 μM (A and C) and 1 mM (B and D)] were incubated with liver microsomes of male (A and B) and female (C and D) rabbits in the presence of an NADPH-generating system. CL, QD, QN, DB and AN denote control, quinidine, quinine, debrisoquine and α-naphthoflavone, respectively. These substances were added to the reaction medium at a concentration of 250 μM. Control was without the inhibitor. Each value represents the mean ± s.e. of duplicate determinations of three different microsomal fractions. a and b show significant differences from the control (non-paired t-test, $P < 0.01$ and $P < 0.05$, respectively).

(male: 1·78 ± 0·12, female 1·19 ± 0·04). As in the case of rats, the activities in male rabbits were significantly higher than those in females. Kinetic analysis using a substrate concentration range of 0·5 to 1000 μM demonstrated that racemic bunitrolol 4-hydroxylation was biphasic, i.e. the reaction was expressed as the summation of two Michaelis-Menten equations. Kinetic parameters (K_m and V_{max}) calculated were K_{m_1} (1·98 ± 0·14 μM) and V_{max_1} [0·73 ± 0·05 nmol min^{-1} (mg protein)$^{-1}$] for a low-K_m system; K_{m_2} (411 ± 66·0 μM) and V_{max_2} [0·79 ± 0·04 nmol min^{-1} (mg protein)$^{-1}$] for a high-K_m system (mean ± s.e., n = 3).

Oxidative metabolism of bunitrolol enantiomers

Enzyme kinetics using bunitrolol enantiomers (0·5 to 1000 μM) were examined in liver microsomes from rabbits of both sexes. As in the case of the oxidative metabolism of racemic bunitrolol, 4-hydroxylations of bunitrolol enantiomers were both analysed to be biphasic (Fig. 1). Kinetic parameters are summarized in Table 1. Significant differences between (+)-bunitrolol and (−)-bunitrolol were observed in-K_m values of both low-K_m and high-K_m systems for males and the value of the low-K_m system for females [(+) > (−)], whereas V_{max} values of the low-K_m system for both sexes were significantly different between the two bunitrolol enantiomers [(+) > (−)]. There was no significant difference in the K_m or V_{max} values between male and female animals.

Metabolic interaction of bunitrolol enantiomers

When bunitrolol enantiomers (each 10 μM) were used as substrates, a ratio of (−)-4-hydroxybunitrolol formation

Table 1. Comparison of kinetic parameters of 4-hydroxylation of bunitrolol enantiomers in liver microsomes from rabbits of both sexes.

Enantiomer	Sex	K_m (μM)		V_{max} (nmol $min^{-1}mg^{-1}$)	
		Low-K_m	High-K_m	Low-K_m	High-K_m
(+)-Bunitrolol	M	7.27 ± 0.42	436 ± 47.3	0.96 ± 0.10	0.78 ± 0.24
	F	10.9 ± 2.70	323 ± 83.8	0.62 ± 0.11	0.90 ± 0.10
(−)-Bunitrolol	M	0.90 ± 0.16[a]	236 ± 26.4[a]	0.55 ± 0.05[a]	1.14 ± 0.16[b]
	F	1.03 ± 0.24[a]	320 ± 84.7	0.34 ± 0.09[a]	0.91 ± 0.11
(−)/(+) Ratio	M	0.12 ± 0.02	0.54 ± 0.03	0.56 ± 0.03	1.46 ± 0.29
	F	0.09 ± 0.01	0.99 ± 0.04	0.54 ± 0.06	1.03 ± 0.17

Each value represents the mean ± s.e. of duplicate determinations of three microsomal fractions from different animals. M and F mean male and female, respectively. Statistical significance was calculated by paired t-test [[a]$P < 0.01$; [b]$P < 0.05$ between (+)-bunitrolol and (−)-bunitrolol, both tails].

Table 2. The rate of clearance (V_{max}/K_m) of bunitrolol 4-hydroxylation in rabbit liver microsomes.

Bunitrolol	Sex	Low-K_m system	High-K_m system
Racemate	M	0.372 ± 0.042	0.0020 ± 0.0004
(+)-Bunitrolol	M	0.134 ± 0.018	0.0018 ± 0.0004
	F	0.057 ± 0.010	0.0029 ± 0.0005
(−)-Bunitrolol	M	0.652 ± 0.109[a]	0.0049 ± 0.0003[a]
	F	0.325 ± 0.059[a,*]	0.0028 ± 0.0004

Each value (mL min^{-1} mg^{-1}) represents the mean ± s.e. of duplicate determinations of three microsomal fractions from different animals. Statistical significance was calculated by paired t-test [[a]$P < 0.01$ from (+)-bunitrolol of corresponding sex, both tails] or non-paired t-test [*$P < 0.05$ from male of (−)-bunitrolol].

(4·80 ± 0·09 nmol min^{-1} mg^{-1}) to (+)-4-hydroxy formation (5·18 ± 0·22) was 0·93. In contrast, when bunitrolol racemate (20 μM) was used as substrate, the ratio of (−)-enantiomer formation (4·45 ± 0·27) to (+)-enantiomer formation (1·82 ± 0·18) was increased to 2·34. Dixon plots demonstrated that addition to the reaction medium of increasing amounts of (+)-bunitrolol to (−)-bunitrolol or vice-versa (Fig. 2) competitively inhibited the 4-hydroxylation of its antipode. Inhibition constants (K_i values) calculated by Dixon plots were 1·98 and 6·25 μM for (−)-bunitrolol and (+)-bunitrolol, respectively.

Inhibition studies

We examined effects of quinine, quinidine (inhibitors of P450-2D enzymes in the rat and man), debrisoquine (a substrate of rat and human P450-2D enzymes), and α-naphthoflavone (an inhibitor of rat P450-1A enzymes) on rabbit liver microsomal 4-hydroxylase activity of bunitrolol enantiomers. None of the inhibitors showed any significant effects in a concentration range from 1 to 10 μM. Quinidine and quinine exhibited some inhibitory effects from 25 μM. Debrisoquine and α-naphthoflavone did not show the inhibition until their concentration was increased to 50 μM. Fig. 3 (upper panels) shows inhibitory effects of various compounds at 250 μM on enantiomeric bunitrolol 4-hydroxylation with a substrate concentration of 30 μM. Quinidine and quinine significantly inhibited enantiomeric bunitrolol 4-hydroxylase activities in both sexes. Debrisoquine and α-naphthoflavone also decreased enantiomeric bunitrolol 4-hydroxylation, but their potencies appear to be smaller than those of quinidine and quinine, particularly in (+)-bunitrolol 4-hydroxylation for both sexes. The effects of these compounds tended to be more potent for (+)-bunitrolol than for (−)-bunitrolol. When the substrate concentration was increased from 30 μM to 1 mM, all of these substances showed significant inhibition in enantiomeric bunitrolol 4-hydroxylation in both sexes, but superiority of quinine and quinidine as inhibitors over debrisoquine and α-naphthoflavone which had been observed at a substrate concentration of 30 μM, disappeared at a substrate concentration of 1 mM (Fig. 3, lower panels). In addition, polyclonal antibodies raised against rat P450-2D and -1A enzymes did not affect bunitrolol 4-hydroxylation in rabbit liver microsomes under the conditions used, whereas the antibodies significantly decreased the same activity by rat liver microsomes (data not shown).

Discussion

Racemic and enantiomeric bunitrolol 4-hydroxylation was found to be kinetically biphasic in this study. Table 2 summarizes the rates of clearance (V_{max}/K_m) of bunitrolol 4-hydroxylation that were calculated from the data in Table 1. The clearance was ca 200-fold higher in the low-K_m system than in the high-K_m system for microsomal 4-hydroxybunitrolol formation from racemic bunitrolol in male rabbits. Similarly, the clearance values in the low-K_m system were 20- to 130-fold those in the high-K_m system, and this tendency was particularly pronounced for (−)-bunitrolol metabolism in male rabbits compared with (+)-bunitrolol metabolism in females (Table 2). This means that P450 enzyme(s) involved in the low-K_m system can play the major part in the oxidative metabolism of bunitrolol at low substrate concentrations, probably corresponding to the in-vivo situation.

We recently reported that the enantioselectivity [(−) > (+)] in bunitrolol metabolism in rat liver microsomes was more pronounced when racemic bunitrolol rather than each bunitrolol enantiomer was employed as substrate (Narimatsu et al 1994a). Table 2 also revealed that the rates of clearance of (−)-bunitrolol 4-hydroxylation were significantly higher than those of (+)-bunitrolol 4-hydroxylation particularly in the low K_m system of both sexes. Moreover, significant sex differences (male > female) were observed in the clearance values for the oxidative metabolism of both bunitrolol enantiomers. Unlike rats that exhibit marked sex differences in the drug metabo-

lism, the rabbit does not generally show sex difference in the metabolism of various drugs including β-blockers.

Inhibition studies showed that the enzyme involved in the low-K_m system was sensitive to quinidine and quinine, which are inhibitors of rat and human P450-2D enzymes (Kobayashi et al 1981; Boobis et al 1990). Sensitivity of rat and human P450-2D enzymes (IC50 < 1 μM) to the inhibitors is much higher than that of the rabbit liver microsomal P450 enzymes that were not sensitive to the inhibitors even at 10 μM in the present study. α-Naphthoflavone, a typical inhibitor of the rat P450-1A subfamily (Johnson et al 1979), also suppressed enantiomeric bunitrolol 4-hydroxylase activity, although it was less potent than quinine and quinidine. This inhibitor also did not exhibit any significant inhibition in a lower concentration range (1–50 μM). However, it should be noted that it is unclear whether these inhibitors for human and rat P450 enzymes have similar specificities for rabbit liver P450 enzymes.

Rabbit liver microsomal bunitrolol 4-hydroxylation was not suppressed by antibodies against rat P450-2D or -1A enzymes, whereas immunoblot analysis showed that two protein bands (molecular weight of 50 000 and 48 500) in male rabbit liver microsomes cross-reacted with antibodies against rat P450-1A and -2D enzymes (unpublished observation). It appears likely that the insensitivities of the antibodies are due to different epitopes between the rat and the rabbit (i.e. species difference). On the basis of these results from the inhibition studies, further detailed enzymological studies are necessary to characterize P450 enzyme(s) involved in bunitrolol hydroxylation in the rabbit liver.

Interestingly, the V_{max} values in the 4-hydroxylation of bunitrolol enantiomers was changed from (+) > (−) in the low-K_m system to (+) < (−) in the high-K_m system particularly in male rabbits (Table 1). On the basis of data in Table 1, the stereoselectivity in the bunitrolol metabolism by P450 is simulated to change from (+) > (−) below the concentration of bunitrolol enantiomers of 8–10 μM to (+) < (−) above this concentration range. We reported previously that P450BTL (P450-2D2) and P450-1A1/2 purified from rat liver microsomes exhibited different stereoselectivity in the ring-hydroxylations of propranolol (Fujita et al 1993). It may be thus interpreted that the stereoselectivity [(+) > (−)] of some P450 enzymes and the inverse selectivity [(+) < (−)] of other P450 enzymes are responsible for the low-K_m and high-K_m systems, respectively, of the 4-hydroxybunitrolol formation from bunitrolol enantiomers in rabbit liver microsomes.

Metabolic interaction studies demonstrated that (+)-bunitrolol 4-hydroxylase activity, which was slightly higher than (−)-bunitrolol 4-hydroxylase activity in the separated incubation, was remarkably suppressed in the presence of its antipode as racemate. In contrast, (−)-bunitrolol 4-hydroxylase activities were similar regardless of the existence of its antipode, resulting in the stereoselectivity reversed from (+) > (−) in enantiomers to (+) < (−) in racemate. Such an interesting change in the stereoselectivity might be due to the difference in K_m values of bunitrolol enantiomers in the low-K_m system; i.e., the Michaelis constant of (+)-bunitrolol (7·27 μM) was 8-fold higher than that of (−)-bunitrolol (0·90 μM).

Bunitrolol is extensively cleared by first-pass metabolism in the liver after its oral administration, so that marked differences in kinetic parameters for 4-hydroxylation of bunitrolol enantiomers may be well reflected in the pharmacokinetics of bunitrolol in the rabbit in-vivo. In this context, detailed pharmacokinetic studies of bunitrolol racemate and enantiomers in the rabbit after oral and intravenous administration of bunitrolol racemate and enantiomers, in combination with in vitro studies, can become a good and useful model of the metabolism and disposition of chiral drugs.

Acknowledgements

We are very grateful to the Nippon Boehringer Ingelheim, Co. (Hyogo, Japan) for the generous gift of bunitrolol and 4-hydroxybunitrolol. This work was supported in part by a grant from the Japan Research Foundation of Clinical Pharmacology to S.N.

References

Boobis, A. R., Sesardic, D., Murray, B. P., Edwards, R. J., Singleton, A. M., Rich, K. J., Murray, S., De La Torre, R., Segura, J., Pelkonen, O., Pasanen, M., Kobayashi, S., Zhi-Guang, T., Davies, D. S. (1990) Species variation in the response of the cytochrome P-450-dependent monooxygenase system to inducers and inhibitors. Xenobiotica 20: 1139-1161

Fujita, S., Matsunaga, T., Masubuchi, Y., Suzuki, T. (1988) Possible mechanism of Sudan III-induced prevention of chemical carcinogenesis in rats. Cancer Res. 48: 254-259

Fujita, S., Umeda, S., Funae, Y., Imaoka, S., Abe, H., Ishida, R., Adachi, T., Masuda, M., Kazusaka, A., Suzuki, T. (1993) Regio- and stereoselective propranolol metabolism by 15 forms of purified cytochrome P-450 from rat liver. J. Pharmacol. Exp. Ther. 264: 226–233

Johnson, E. F., Schwab, G. E., Muller-Eberhard, U. (1979) Multiple forms of cytochrome P-450: catalytic differences exhibited by two homogenous forms of rabbit cytochrome P-450. Mol. Pharmacol. 15: 708–718

Kobayashi, S., Murray, S., Watson, D., Sesardic, D., Davies, D. S., Boobis, A. R. (1981) The specificity of inhibition of debrisoquine 4-hydroxylase activity in quinidine and quinine in the rat is the inverse of that in man. Biochem. Pharmacol. 38: 879–885

Kono, T., Ohiwa, Y., Nagakura, A., Kobayashi, S., Matsumura, R., Kohei, H. (1987) Study on blood concentration and safety of single and repeated administration of long-acting bunitrolol (Ko1366PL) 20 mg in healthy volunteers. Yakuri to Chiryou 15: 131–148

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275

Narimatsu, S., Huang, Y., Mizukami, T., Masubuchi, Y., Suzuki, T. (1994a) Development of a high-performance liquid chromatographic method for the analysis of enantiomer/enantiomer interaction in oxidative metabolism of bunitrolol in rat liver microsomes. Anal. Biochem. 222: 256–261

Narimatsu, S., Masubuchi, Y., Hosokawa, S., Ohmori, S., Kitada, M., Suzuki, T. (1994b) Involvement of cytochrome P4502D subfamily in human liver microsomal bunitrolol 4-hydroxylation. Biol. Pharm. Bull. 17: 803–807

Omura, T., Sato, R. (1964) The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. J. Biol. Chem. 239: 2370–2378

Suzuki, T., Rikihisa T. (1979) Studies on absorption, distribution, metabolism and excretion of bunitrolol, a β-adrenergic blocking agent in rats and mice. Arzneim. Forsch. Drug Res. 29: 1707–1715

Suzuki, T., Narimatsu, S., Fujita, S., Masubuchi, Y., Umeda, S. (1991) Impairment of bunitrolol 4-hydroxylase activity in liver microsomes of Dark Agouti rats. Biochem. Pharmacol. 42: 2241–2244

Suzuki, T., Narimatsu, S., Fujita, S., Masubuchi, Y., Umeda, S., Imaoka, S., Funae, Y. (1992) Purification and characterization of a cytochrome P-450 isozyme catalyzing bunitrolol 4-hydroxylation in liver microsomes of male rats. Drug Metab. Dispos. 20: 367–373

Yamaoka, K., Tanigawara, Y., Nakagawa, T., Uno, T. (1981) A pharmacokinetic analysis program (MULTI) for microcomputer. J. Pharmacobiodyn. 4: 879–885