Effects of Pretreatment of Hep G2 Cells with β-Naphthoflavone on Cytotoxicity of Propranolol and its Active Metabolite 4-Hydroxypropranolol

Junko Miyano,[a] Harumi Motoyama,[a] Masako Fukuoka,[a] Shigeo Yamamoto,[b] Shizuo Narimatsu,*[,a] Kenichiro Ogura,[c] Tadashi Watabe,[c,d] Masuhiro Nishimura,[e] Nobuhiko Ueda,[e] and Shinsaku Naito[e]

[a]Laboratories of Health Chemistry and [b]Biomolecular Science, Faculty of Pharmaceutical Sciences, Okayama University, 1–1–1 Tsushima-naka, Okayama 700–8530, Japan, [c]Department of Drug Metabolism and Molecular Toxicology, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432–1 Horinouchi, Hachioji, Tokyo 192–0392, Japan, [d]Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930–0194, Japan, and [e]Division of Pharmacology, Drug Safety and Metabolism, Otsuka Pharmaceutical Factory, Inc., 115 Kuguhara, Tateiwa, Naruto, Tokushima 772–8601, Japan

(Received April 7, 2003; Accepted April 22, 2003)

Cytotoxicities of propranolol (PL) and its active metabolite, 4-hydroxypropranolol (4-OH-PL), were examined in a human hepatoma cell line, Hep G2. Hep G2 cells were cultured in the presence of β-naphthoflavone (BNF, 25 or 50 μM), lansoprazole (LPZ, 25 or 50 μM) or 0.5% dimethylsulfoxide (vehicle) for 48 hr. The cells were harvested, and microsomal and cytosolic fractions were prepared by differential centrifugation methods. Various enzyme activities were determined as follows: microsomal 7-ethoxyresorufin (ER) *O*-deethylation as a CYP1A1 index, microsomal phenacetin (PN) *O*-deethylation as a CYP1A2 index, microsomal and cytosolic *p*-nitrophenyl acetate (NPA) hydrolysis as a carboxylesterase index and cytosolic 4-OH-PL sulfation as a sulfotransferase index. The pretreatment of Hep G2 cells with LPZ or BNF increased microsomal ER *O*-deethylase activities, and the potency of BNF was much higher than that of LPZ. Cytosolic 4-OH-PL sulfation was also elevated by the pretreatment with BNF but not with LPZ. Microsomal PN *O*-deethylase activity was not detectable in either the control or BNF-pretreated group under the conditions used. Microsomal and cytosolic NPA hydrolase activities were similar between the control and the BNF-pretreated groups. Cytotoxicities of PL and 4-OH-PL were attenuated in BNF-pretreated Hep G2 cells compared to non-pretreated Hep G2 cells. These results suggest that increased activities of microsomal CYP1A1 and cytosolic sulfotransferases by pretreatment with BNF may contribute to the attenuating the cytotoxicity of PL and 4-OH-PL in Hep G2 cells, at least in part.

Key words —— propranolol, 4-hydroxypropranolol, cytotoxicity, Hep G2 cell, β-naphthoflavone, sulfation

INTRODUCTION

Propranolol (PL) is a typical β-adrenoceptor blocking agent often prescribed clinically. Following oral administration to humans, PL is extensively biotransformed to various oxidative metabolites such as 4-hydroxypropranolol (4-OH-PL), 5-OH-PL and *N*-desisopropylpropranolol (NDP).[1] These oxidative metabolites and also PL itself are excreted into urine or bile mainly in conjugated forms, *i.e.*, glucuronides or sulfates.[2] The oxidation of PL is mediated by hepatic cytochrome P450 (CYP) enzymes, and the formation of 4-OH-PL is mainly catalyzed by CYP2D6, whereas that of NDP is mainly by CYP1A2 in human liver microsomes.[3]

It was reported that repeated administration of PL to patients resulted in increased systemic availability of PL.[4,5] Although PL itself was demonstrated to inhibit CYP-dependent drug- metabolism,[6,7] an inhibitory mechanism was proposed in which a reactive metabolic intermediate of PL covalently bound to CYP species catalyzing PL ring-hydroxylations.[8,9] 4-OH-PL, one of major metabolites of PL, is a pharmacologically active metabolite whose β-blocking activity is almost equivalent to the parent compound.[10] 4-OH-PL is also a chemically reactive metabolite that covalently binds to proteins after

*To whom correspondence should be addressed: Laboratory of Health Chemistry, Faculty of Pharmaceutical Sciences, Okayama University, 1–1–1 Tsushima-naka, Okayama 700–8530, Japan. Tel. & Fax: +81-86-251-7942; E-mail address: shizuo@pharm.okayama-u.ac.jp

conversion to a quinone metabolite, which may be one of the causes of inactivation of CYP2D enzymes by PL in the rat both *in vitro* and *in vivo*.[11–14]

In the course of our studies on the metabolic fate of 4-OH-PL, an appropriate cell system was required to screen the cellular toxicities of PL and 4-OH-PL. Hep G2 cells are a human hepatoma cell line that have various functions also observed in normal hepatocytes. Steiner *et al*.[15] found that the CYP1A enzyme activities were elevated in Hep G2 cells pretreated with 3-methylcholanthrene. Moreover, Walle *et al*.[16] found high sulfation activities towards some substrates in Hep G2 cells and proposed it as a good cell line for drugs that are subject to sulfation as a major metabolic pathway. In the present study, the Hep G2 cell line was thus employed to screen the cytotoxicity of PL and its major metabolite, 4-OH-PL, and the effects of the pretreatments with inducers of CYP1A enzymes on some drug-metabolizing enzymes in Hep G2 cells were examined.

MATERIALS AND METHODS

Chemicals —— PL hydrochloride, lansoprazole (LPZ), ethoxyresorufin (ER), resorufin, phenacetin (PN), acetoaminophene, *p*-nitrophenyl acetate (NPA), adenosine 3′-phosphate 5′-phosphosulfate, penicillin-streptomycin solution, and Williams' medium E were obtained from Sigma Chemicals (St. Louis, MO, U.S.A.) and trypan blue was from Nacalai Tesque (Kyoto, Japan). 4-OH-PL hydrochloride and 4-hydroxybunitrolol (4-OH-BTL) hydrochloride were supplied by Sumitomo Chemical Co. (Osaka, Japan) and Nippon Bohringer Ingelheim Co. (Hyogo, Japan), respectively. Fetal bovine serum (FBS) was purchased from Gibco BRL (Grand Island, NY, U.S.A.). Other reagents or solvents used were of the highest quality commercially available.
Cell Culture —— Hep G2 cells were obtained from Riken Cell Bank (Ibaraki, Japan). Cells were cultured in Williams' medium E supplemented with 10% FBS and penicillin (100 units/ml)-streptomycin (100 mg/ml) at 37°C in a humidified 5% CO_2 atmosphere. When cells in 150 mm dishes were about 90% confluent, they were treated with LPZ or β-naphthoflavone (BNF) (25 and 50 μM final concentrations) dissolved in dimethylsulfoxide (DMSO) (0.5% final volume) or the same volume of DMSO for 48 hr. Following the treatment, the cells were washed with the culture medium containing no drugs, and further cultured for 24 hr without drugs. The cells were then suspended in ice-cold 100 mM potassium phosphate buffer (pH 7.4), frozen in liquid nitrogen and thawed in a 37°C water bath followed by homogenization with a Potter Elevehjem-type glass homogenizer and teflon pestle on ice. This process (freezing, thawing and homogenizing) was repeated five times, the final homogenate was centrifuged at $9000 \times g$ for 20 min at 4°C, and the supernatant was centrifuged at $105000 \times g$ for 1 hr at 4°C. The supernatant obtained was used as the cytosolic fraction. The resultant pellet was resuspended in 100 mM potassium phosphate buffer (pH 7.4) and used as the microsomal fraction.
Cytotoxicity of PL and 4-OH-PL —— PL or 4-OH-PL was dissolved in distilled water, and added to the cell culture medium (50 and 100 μM final concentrations). Hep G2 cells were cultured in the medium containing BNF (50 μM) for 48 hr, and then the medium was changed to a fresh one containing no inducer, followed by further 24 hr of culture without BNF. The culture medium was then changed to the medium containing PL or 4-OH-PL described above, and cultured for 72 hr. The Hep G2 cells cultured with PL or 4-OH-PL were taken at different time intervals (0, 24, 48 and 72 hr), and the cell viability was measured by exclusion of trypan blue.
Enzyme Assays —— Microsomal ER *O*-deethylase activity as an index of CYP1A1 was measured by the method of Sinjari *et al*.[17] Microsomal PN *O*-deethylase activity as an index of CYP1A2 was assayed according to the method of Sesardic *et al*.[18] Microsomal and cytosolic PNA hydrolase activities as an index of carboxylesterase were determined by the method of Forkert and Lee.[19] Cytosolic sulfotransferase activities toward 4-OH-PL were measured according to a published HPLC method.[20] In the 4-OH-PL sulfation assay, activities were calculated using peak height ratios of 4-OH-PL sulfate to 4-OH-BTL as an internal standard because of the lack of an authentic standard. Protein concentrations were measured by the method of Lowry *et al*.[21]

RESULTS AND DISCUSSION

Both LPZ[22] and BNF[23] are known to be typical inducers of CYP1A enzymes in human hepatocytes. Pretreatment of Hep G2 cells with LPZ (25 and 50 μM) increased ER *O*-deethylase activity by 4.2- to 4.8-fold that of the control cells pretreated with

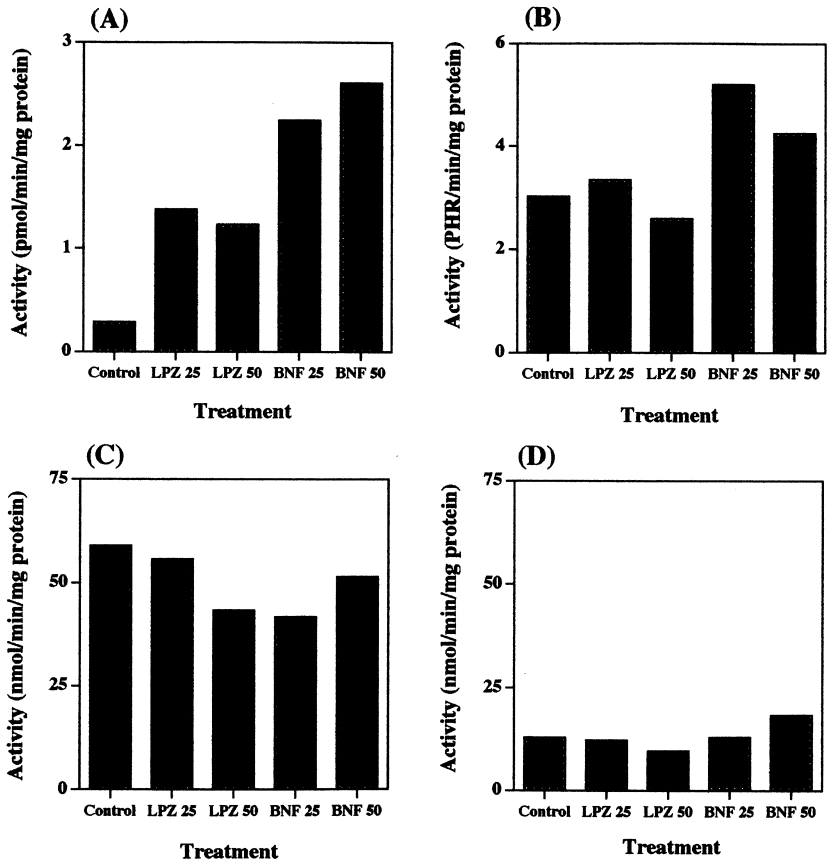

Fig. 1. Effetcs of Pretreatment with LPZ and BNF on Various Drug-Metabolizing Enzyme Activities in Hep G2 Cells

Hep G2 cells were cultured with LPZ or BNF (25 and 50 μM) for 48 hr, followed by further culture in the medium without the drugs for 24 hr. Microsomal and cytosolic fractions were prepared from Hep G2 cells, and various drug-metabolizing enzyme activities were assayed. (A) microsomal ER *O*-deethylation, (B) Cytosolic 4-OH-PL sulfation, (C) Microsomal NPA hydrolysis, (D) Cytosolic NPA hydrolysis. In the 4-OH-PL sulfation assay, activities were calculated using peak height ratios of 4-OH-PL sulfate to 4-OH-BTL as an internal standard because of the lack of an authentic standard. Each value is the mean of two determinations.

the vehicle (0.5% DMSO) only. BNF markedly increased the activity by 7.2- to 9.0-fold that of the control, whose effect was much higher than that of LPZ. These results were consistent with those of Steiner *et al.*[16] who showed that the pretreatment of Hep G2 cells with 3-methylcholanthrene (3-MC) increased the formation of NDP from PL, which was mainly mediated by CYP1A enzymes.

Sulfation activity toward 4-OH-PL in BNF-pretreated cells increased by 1.4- to 1.7-fold that of the control, although the activity was not changed by the treatment with LPZ (Fig. 1B). Microsomal or cytosolic NPA hydrolysis activities were not changed by the pretreatment with LPZ or BNF under the conditions used in the present study (Figs. 1C and D). In addition, no PNT *O*-deethylase activities could be detected either in the control or inducer-treated cells.

In preliminary experiments, 10 μM of BNF did not show such a clear increasing effect on 4-OH-PL sulfation. Since the highest inducing effect on ER *O*-deethylation was observed in Hep G2 cells pretreated with 50 μM BNF, the time course of ER *O*-deethylation and 4-OH-PL sulfation was then examined following the removal of BNF from the culture medium of Hep G2 cells pretreated with 50 μM BNF for 48 hr. As shown in Fig. 2A, the highest ER *O*-deethylase activity (17.0-fold that of 0 hr control) was observed in the cells cultured for 24 hr after the removal of BNF from the culture medium, and then the activity decreased quickly. Following the removal of BNF from culture, on the other hand, 4-OH-PL sulfation reached a peak at 48 hr (2.9-fold that of 0 hr control) and did not decrease so much even after culture for 72 hr (2.5-fold that of 0 hr control) (Fig. 2B).

On the basis of the results obtained, the cytotoxicity of PL or 4-OH-PL (50 and 100 μM) was exam-

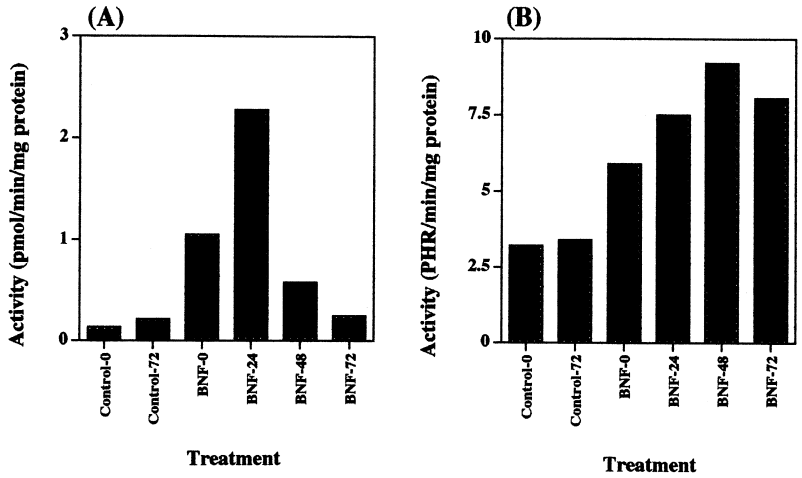

Fig. 2. Time Course of Microsomal ER *O*-Deethylation (A) and Cytosolic 4-OH-PL Sulfation Following the Removal of BNF from Cultured Hep G2 Cells

Hep G2 cells were cultured in the medium containing BNF (50 μM) for 48 hr and the culture medium was changed to fresh BFN-free medium, followed by further culturing for 72 hr. Then, portions of the Hep G2 cells were taken at various time intervals (0, 24, 48 and 72 hr), and their microsomal and cytosolic fractions were prepared. In the 4-OH-PL sulfation assay, activities were calculated using peak height ratios of 4-OH-PL sulfate to 4-OH-BTL as an internal standard because of the lack of an authentic standard. Each value is the mean of two determinations.

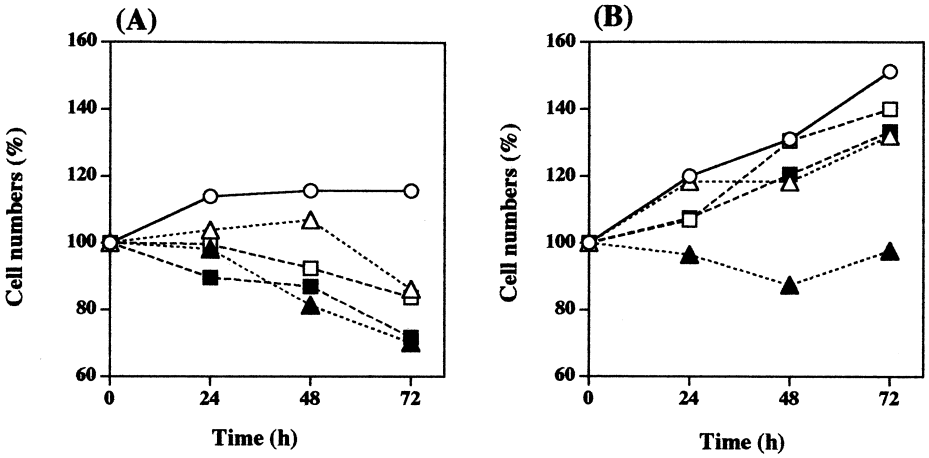

Fig. 3. Change in Cytotoxicities of PL and 4-OH-PL in Non-Pretreated (A) and BNF-Pretreated Hep G2 Cells (B)

Hep G2 cells were cultured with BNF (50 μM) or 0.5% DMSO for 48 hr, and PL or 4-OH-PL (50 and 100 μM) or distilled water was added, followed by further culture for 72 hr. Cell viabilities were measured by the exclusion of trypan blue method at various time intervals (0, 24, 48 and 72 hr). Open circle, control (distilled water instead of PL or 4-OH-PL was added); open triangle, 50 μM PL; closed triangle, 100 μM PL; open square, 50 μM 4-OH-PL; closed square, 100 μM 4-OH-PL. Each value is the mean of two determinations. Cell numbers at 0 hr: 1.51×10^7 for the control cells pretreated with vehicle (0.5% DMSO); 0.60×10^7 for the cells pretreated with 50 μM BNF.

ined in Hep G2 cells which had been cultured in the presence of 50 μM BNF for 48 hr followed by further culture for 24 hr without BNF. In the control Hep G2 cells without the inducer pretreatment (Fig. 3A), both PL and 4-OH-PL decreased cell viability in a concentration-dependent manner. The percentage numbers of living cells to those of the control group without PL or 4-OH-PL (1.74×10^7 cells, taken as 100%) at 72 hr of culture were 74.6% (50 μM PL), 72.6% (50 μM 4-OH-PL), 62.1% (100 μM 4-OH-PL) and 60.9% (100 μM PL). Cell toxicity of PL and 4-OH-PL was attenuated by the pretreatment of Hep G2 cells with BNF (Fig. 3B). The percentage numbers of living cells to those of the BNF-pretreated control group without PL or 4-OH-PL (0.60×10^7 cells, taken as 100%) at 72 hr of culture were 93.0% (50 μM PL), 89.1% (100 μM 4-OH-PL), 87.4% (50 μM 4-OH-PL) and 64.2% (100 μM PL). That is, BNF was found to protect cells from the toxicity of PL or 4-OH-PL at all concentra-

tions tested except for 100 μM PL.

In the present study, the addition of PL or 4-OH-PL to non-treated Hep G2 cells decreased the viabilities to 60–70% that of the control cells to which only the vehicle (distilled water) instead of PL or 4-OH-PL was added. 4-OH-PL is known to be not only a pharmacologically active metabolite[10] but also a chemically reactive metabolite,[11–14] so that the cytotoxicity of 4-OH-PL in Hep G2 cells might be comparable to the parent compound. The pretreatment of Hep G2 cells with BNF attenuated the cytotoxicity of both PL and 4-OH-PL.

We demonstrated previously that the oxidative metabolism of PL was mainly mediated by CYP2D6 as a ring-hydroxylase and CYP1A2 as an *N*-desisopropylase in human liver microsomal fractions.[3] Recombinant human CYP1A1 and CYP1A2 were reported to oxidize PL efficiently.[24] However, the content of CYP1A1 is usually very low in the human liver.[25] In the course of performing the present study, Walle *et al*.[26] reported that CYP1A1 and glucuronosyltransferase UGT1A1 in Hep G2 were induced by various flavonoids. Furthermore, PN *O*-deethylase activity, a human CYP1A2 index, was not observed even in BNF-pretreated Hep G2 cells in this study. Therefore, it seems likely that PL added to the culture medium might be oxidized mainly by CYP1A1 induced in BNF-pretreated Hep G2 cells.

The major metabolic pathway of 4-OH-PL, on the other hand, is thought to be the conjugation (glucuronidation and sulfation) of a phenolic hydroxyl group in BNF-pretreated Hep G2 cells forming 4-OH-PL sulfate, although some amounts of 4-OH-PL may be oxidized to *N*-desisopropylated 4-OH-PL by CYP1A1 induced by BNF. The conjugation of 4-OH-PL by glucuronosyltransferases or sulfotransferases is thought to be a detoxication pathway, whereas the oxidation of PL by CYP1A1 does not seem to be a simple detoxication pathway. These circumstances may have caused the the low viability profile in BNF-pretreated cells to which 100 μM PL was added.

The present study demonstrated that pretreatment of Hep G2 cells with BNF increased 4-OH-PL sulfation as well as ER *O*-deethylation, and the toxicity of PL and 4-OH-PL in Hep G2 cells was attenuated by BNF-pretreatment. These results suggest that increased activities of microsomal CYP1A1 and cytosolic sulfotransferases by the pretreatment with BNF may contribute to attenuating the cytotoxicity of PL and 4-OH-PL to Hep G2 cells, at least in part. Further studies are necessary to elucidate which sulfotransferase species are induced by BNF in Hep G2 cells.

REFERENCES

1) Marathe, P. H., Shen, D. D. and Nelson, W. L. (1994) Metabolic kinetics of pseudoracemic propranolol in human liver microsomes. *Drug Metab. Dispos.*, **22**, 237–247.
2) Walle, T., Walle, U. K., Cowart, T. D., Conradi, E. C. and Gaffney, T. E. (1987) Selective induction of propranolol metabolism by smoking: Additional effects on renal clearance of metabolite. *J. Pharmacol. Exp. Ther.*, **241**, 928–933.
3) Masubuchi, Y., Hosokawa, S., Horie, T., Ohmori, S., Kitada, M., Suzuki, T. and Narimatsu, S. (1994) Cytochrome P-450 isozymes involved in propranolol metabolism in human liver microsomes: The role of CYP-2D6 as ring-hydroxylase and CYP1A2 as N-desisopropyrase. *Drug Metab. Dispos.*, **22**, 909–915.
4) Evans, G. H. and Shand, D. G. (1973) Disposition of propranolol. V. Drug accumulation and steady state concentrations during chronic oral administration in man. *Clin. Pharmacol. Ther.*, **14**, 487–493.
5) Straka, R. J., Lalonde, R. L., Pieper, J. A., Bottorff, M. B. and Mirvis, D. M. (1987) Non-linear pharmacokinetcis of unbound propranolol after oral administration. *J. Pharm. Sci.*, **76**, 521–524.
6) Bax, D. N. S., Lennard, M. S. and Tucker, G. T. (1983) Inhibition of antipyrine metabolism by beta-adrenocetor antagonists. *Br. J. Pharmacol.*, **12**, 779–784.
7) Suzuki, T., Ishida, R., Matsui, S., Masubuchi, Y. and Narimatsu, S. (1993) Kinetic analysis of metabolic inhibition between lidocaine and propranolol in rat liver microsomes. *Biochem. Pharmacol.*, **45**, 1528–1530.
8) Schneck, D. W. and Prichard, J. F. (1981) The inhibitory effect of propranolol pretreatment on its own metabolism in the rat. *J. Pharmacol. Exp. Ther.*, **218**, 575–581.
9) Shaw, L., Lennard, M. S., Tucker, G. T., Bax, N. D. S. and Woods, H. F. (1987) Irreversible binding and metabolism of propranolol by human liver microsomes — relationship to polymorphic oxidation. *Biochem. Pharmacol.*, **36**, 2283–2288.
10) Fitzgerald, D. J. and O'Donnell, S. R. (1971) Pharmacology of 4-hydroxypropranolol. *Br. J. Pharmacol.*, **43**, 222–235.

11) Masubuchi, Y., Narimatsu, S., Hosakawa, S. and Suzuki, T. (1994) Role of the CYP2D subfamily in metabolism-dependent propranolol binding to liver microsomal protein in rats. *Biochem. Pharmacol.*, **48**, 1891–1898.
12) Narimatsu, S., Watanabe, T., Mochida, M., Masubuchi, Y., Horie, T., Kumagai, Y., Cho, A. K., Imaoka, S., Funae, Y., Ishikawa, T. and Suzuki, T. (1995) Characterization of a chemically reactive propranolol metabolite that bind to microsomal proteins of rat liver. *Chem. Res. Toxicol.*, **8**, 721–728.
13) Narimatsu, S., Arai, T., Watanabe, T., Horie, T., Suzuki, T., Ishikawa, T., Tsutsui, M., Kumagai, Y. and Cho, A. K. (1997) Covalent binding of a reactive metabolite derived from propranolol and its active metabolite 4-hydroxypropranolol to hepatic microsomal proteins in the rat. *Chem. Res. Toxicol.*, **10**, 289–295.
14) Narimatsu, S., Arai, T., Masubuchi, Y., Horie, T., Hosokawa, M., Ueno, K., Kataoka, H., Yamamoto, S., Ishikawa, T. and Cho, A. K. (2001) Inactivation of Rat Cytochrome P450 2D Enzyme by a Further Metabolite of 4-Hydroxypropranolol, the Major and Active Metabolite of Propranolol. *Biol. Pharm. Bull.*, **24**, 988–994.
15) Steiner, A., Walle, U. K. and Walle, T. (1992) Induction of propranolol metabolism in the Hep G2 human hepatoma cell line. *J. Pharm. Pharmacol.*, **44**, 611–614.
16) Walle, T., Walle, U. K., Shwed, J. A., Thornburg, K. R., Mathis, C. E. and Pesola, G. R. (1994) Human phenol sulfotransferases: chiral substrates and expression in Hep G2 cells. *Chem.-Biol. Interact.*, **92**, 47–55.
17) Sinjari, T., Trnwall, U. and Darnerud, P. O. (1993) Induction of 7-ethoxyresorufin-O-deethylase (EROD) activity in mice foetuses by the PCB-congener 3,3′,4,4′-tetrachlorobiphenyl. *Xenobiotica*, **23**, 107–114.
18) Sesardic, D., Edwards, R. J., Davies, D. S., Thomas, P. E., Levin, W. and Boobis, A. R. (1990) High affinity phenacetin O-deethylase is catalyzed by cytochrome P450d (P4501A2) in the liver of rat. *Biochem. Pharmacol.*, **39**, 489–498.
19) Forkert, P. G. and Lee, R. P. (1997) Metabolism of ethyl carbamate by pulmonary cytochrome P450 and carboxylesterase isozymes: Involvement of CYP2E1 and hydrolase A. *Toxicol. Appl. Pharmacol.*, **146**, 245–254.
20) Narimatsu, S., Kobayashi, N., Asaoka, K., Masubuchi, Y., Horie, T., Hosokawa, M., Ishikawa, T., Ohmori, S., Kitada, M., Miyano, J., Kataoka, H. and Yamamoto, S. (2001) High-performance liquid chromatographic analysis of the sulfation of 4-hydroxypropranolol enantiomers by monkey liver cytosol. *Chirality*, **13**, 140–147.
21) Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.
22) Runge, D., Kohler, C., Kostrubsky, V. E., Jager, D., Lehmann, T., Runge, D. M., May, U., Stolz, D. B., Strom, S. C., Fleig, W. E. and Michalopoulos, G. K. (2000) Induction of cytochrome P450 (CYP)1A1, CYP1A2, and CYP3A4 but not of CYP2C9, CYP2C19, multidrug resistance (MDR-1) and multidrug resistance associated protein (MRP-1) by prototypical inducers in human hepatocytes. *Biochem. Biophys. Res. Commun.*, **273**, 333–341.
23) Masubuchi, N., Li, A. P. and Okazaki, O. (1998) An evaluation of the cytochrome P450 induction potential of pantoprazole in primary human hepatocytes. *Chem. Biol. Interact.*, **114**, 1–13.
24) Ching, M. S., Bichara, N., Blake, C. L., Chabrial, H., Tukey, R. H. and Smallwood, R. A. (1996) Propranolol 4- and 5-hydroxylation and N-desisopropylation by cloned human cytochrome P4501A1 and P4501A2. *Drug Metab. Dispos.*, **24**, 692–694.
25) Rendic, S. and Di Carlo, F. J. (1997) Human cytochrome P450 enzymes: A status report summarizing their reactions, substrates, inducers, and inhibitors. *Drug Metab. Rev.*, **29**, 413–580.
26) Walle, T., Otake, T., Galijatovic, A., Ritter, J. K. and Walle, U. K. (2002) Induction of UDP-glucuronosyltransferase UGT1A1 by the flavonoid chrysin in the human hepatoma cell line Hep G2. *Drug Metab. Dispos.*, **28**, 1077–1082.