Comparison of the Effects of Various Inducers on 7-Alkoxycoumarin O-Dealkylase Activities in Liver Microsomes

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Abstract—The effects of six inducers and malotilate on 7-alkoxycoumarin O-dealkylase activities in rat liver microsomes were examined. Phenobarbital (PB) (100 mg/kg) was administered intraperitoneally to rats for 6 days; 3-methylcholanthrene (3-MC) (40 mg/kg), β -naphthoflavone (β -NF) (40 mg/kg), isosafrole (150 mg/kg) and polychlorinated biphenyls (PCB) (100 mg/kg) were administered intraperitoneally for 3 days; isoniazid (INH) (50 mg/kg) was administered intraperitoneally for 10 days; and malotilate (500 mg/kg) was administered orally for 3 days. The O-dealkylase activities toward 7-methoxycoumarin (7-MC), 7-ethoxycoumarin (7-EC) and 7-propoxycoumarin (7-PC) were examined 24 hr after the final administration of the drugs. The ratios of 7-EC O-deethylase and 7-PC O-depropylase to 7-MC O-demethylase activity in the control and six inducer-treated groups were compared. The ratios in the groups treated with the six compounds, each of which induces a different form(s) of cytochrome P-450 (P-450), were clearly different from each other. Therefore, the measurement of 7-alkoxycoumarin O-dealkylase activities should be extremely useful for the routine determination of the molecular species of P-450. On the other hand, the ratio in the malotilate-treated group was different from that in any other inducer-treated group, so that there might be a possibility that malotilate induced a form(s) of P-450 that is different from any of the already known species.

Many hydrophobic compounds, including drugs, chemicals, environmental pollutants and carcinogens, are biotransformed into more hydrophilic metabolites through the mixed-function oxidase system in liver microsomes, in which cytochrome P-450 (P-450) plays an important role as a key enzyme (1).

It has been recognized that P-450 consists of multiple species and that various compounds induce a different form(s) of P-450, each of which has a characteristic substrate specificity to some extent (2, 3). Levin et al. (4-9) have systematically purified various forms of P-450 from rats, which have been designated as P-450a-j.

Recently, the measurement of 7-alkoxycoumarin O-dealkylase activities (7-methoxycoumarin (7-MC) O-demethylation, 7-ethoxycoumarin (7-EC) O-deethylation, 7-

propoxycoumarin (7-PC) O-depropylation and 7-butoxycoumarin (7-BC) O-debutylation) has been reported to be useful for investigating the molecular multiplicity of P-450. Matsubara et al. (10) demonstrated that all these O-dealkylase activities in rat liver microsomes were stimulated by the treatment of animals with phenobarbital (PB); in contrast, treatment with 3-methylcholanthrene (3-MC) or β -naphthoflavone (β -NF) markedly increased 7-EC O-deethylase, 7-PC O-depropylase and 7-BC O-debutylase activities, but not 7-MC O-demethylase activity. Kasahara et al. (11) investigated the effects of D-galactosamine and carbon tetrachloride (CCl_4) on 7-alkoxycoumarin O-dealkylase activities in rat liver microsomes. They reported that the ratio of 7-EC O-deethylase and 7-PC O-depropylase to 7-

MC O-demethylase activity was not altered by the D-galactosamine treatment, but decreased by the CCl_4 treatment, although all these O-dealkylase activities were reduced by either pretreatment. They both emphasized that the measurement of 7-alkoxycoumarin O-dealkylase activities was applicable for the routine determination of the molecular species of P-450. Kamataki et al. (12) referred to the effects of isosafrole and spironolactone in their report concerning sex differences in 7-alkoxycoumarin O-dealkylase activities. However, there are no reports, to our knowledge, concerning the effect of any other inducer on 7-alkoxycoumarin O-dealkylase activities.

In the present study, we examined the effects of six inducers, PB, 3-MC, β -NF, isosafrole, polychlorinated biphenyls (PCB) and isoniazid (INH), each of which induces a different form(s) of P-450 (2, 3), on 7-alkoxycoumarin O-dealkylase activities and compared them with each other. We also measured these O-dealkylase activities in rats treated with malotilate, a new type of inducer (13, 14), and compared them with those in the groups treated with the other six inducers.

Materials and Methods

Animals and treatments: Male Sprague-Dawley rats, each weighing 200 g, were maintained on a standard pellet diet and water ad libitum. PB was dissolved in saline and administered intraperitoneally to the rats at a dose of 100 mg/kg, once a day for 6 days. 3-MC, β -NF, isosafrole and PCB were dissolved in olive oil and administered intraperitoneally for 3 days at a dose of 40 mg/kg, 40 mg/kg, 150 mg/kg and 100 mg/kg, respectively. INH was diluted with saline and administered intraperitoneally at a dose of 50 mg/kg for 10 days. Malotilate was dissolved in olive oil and administered orally at a dose of 500 mg/kg for 3 days. The animals were sacrificed 24 hr after the final administration of the drugs. Untreated rats were used as the control. They were fasted 18 hr prior to killing, but had free access to tap water.

Preparation of microsomes: Rat liver microsomes were prepared as follows: Excised livers were thoroughly perfused with

cold 0.15 M KCl and homogenized in 4-fold volumes of 0.15 M KCl solution containing 10 mM EDTA using a Potter-type Teflon glass homogenizer. The homogenate was centrifuged at $10,000\times g$ for 15 min in a refrigerated centrifuge (Kubota, KR/20000). The supernatant was then centrifuged at $105,000\times g$ for 60 min in a preparative ultracentrifuge (Hitachi, 70P-72). The pellet of microsomes was suspended in the homogenizing solution in the homogenizer and centrifuged again as described above. The resulting pellet was suspended in 20 mM potassium phosphate buffer (pH 7.4) containing 15% glycerol. These operations were performed at 0–4°C.

Assays of drug metabolizing activity: The content of P-450 was measured by the method of Omura and Sato (15). The content of cytochrome b_5 (b_5) and the activity of NADPH-cytochrome c reductase were assayed by the method of Omura and Takesue (16). The microsomal protein content was measured by the method of Lowry et al. (17).

The O-dealkylase activities toward 7-MC, 7-EC and 7-PC were determined by a direct fluorometric measurement of 7-hydroxycoumarin produced according to the method of Ullrich and Weber (18). The substrates, 7-MC, 7-EC and 7-PC, were dissolved in 1 M Tris-HCl buffer (pH 7.6) to give 10^{-3} M solutions. The incubation mixture consisted of microsomes, 10^{-4} M substrate and 10^{-4} M NADPH in 1.0 ml of 0.1 M Tris-HCl buffer (pH 7.6). The reaction was started by adding NADPH ($10\text{ }\mu\text{l}$, 10^{-2} M), and the mixture was incubated aerobically at 37°C. The increase in the fluorescence intensity with time was measured at 460 nm with an excitation of 372 nm using a fluorescence spectrophotometer (Hitachi, 650-60).

Chemicals: The drugs used were as follows: PB (Tokyo Kasei Kogyo Co., Tokyo), 3-MC and β -NF (Sigma Chemical Co., St. Louis, MO, U.S.A.), isosafrole (Nakarai Chemicals, Kyoto), PCB (Kanechlor KC-500, Gasukuro Kogyo Co., Tokyo), INH (Iscotin® (Inj.), Daiichi Pharmaceutical Co., Tokyo). Malotilate was a generous gift from Daiichi Pharmaceutical Co., Tokyo. 7-EC and 7-MC were purchased from Nakarai Chemicals (Kyoto) and Aldrich Chemical Co.

(Milwaukee, WI, U.S.A.), respectively. 7-PC was synthesized at Sorl Laboratory (Aoyama-cho, Mie, Japan).

Statistical analysis: Significance of difference was determined by Student's *t*-test for unpaired variables.

Results

Contents of P-450 and b_5 and the activity of NADPH-cytochrome *c* reductase in rats treated with various inducers: The effects of the six inducers and malotilate on the contents of P-450 and b_5 and the activity of NADPH-cytochrome *c* reductase (/mg microsomal protein) are shown in Table 1.

The P-450 content was noticeably increased to 155–205% of the control level in the PB-, 3-MC-, β -NF-, isosafrole- and PCB-treated groups. In contrast, it was decreased to 73.2% of the control level ($P < 0.01$) by the malotilate treatment and not significantly affected by the INH treatment. The b_5 content was noticeably increased to 163–233% of the control level in every inducer-treated group

and the malotilate-treated group. The activity of NADPH-cytochrome *c* reductase was enhanced by the PB, isosafrole and malotilate treatments to 160, 267 and 192% of the control level, respectively. In contrast, the treatments with 3-MC, β -NF, PCB and INH did not significantly affect the activity of NADPH-cytochrome *c* reductase.

O-Dealkylase activities toward 7-MC, 7-EC and 7-PC in rats treated with various inducers: The effects of the six inducers and malotilate on the O-dealkylase activities toward 7-MC, 7-EC and 7-PC (/nmol P-450) are shown in Figs. 1, 2 and 3, respectively. The ratio of 7-EC O-deethylase and 7-PC O-depropylase to 7-MC O-demethylase activity in each group is shown in Table 2.

The ratio of 7-EC O-deethylase and 7-PC O-depropylase to 7-MC O-demethylase activity in the control group was 1.00:1.08:0.13. In the PB-treated group, the O-dealkylase activities toward 7-MC, 7-EC and 7-PC were significantly increased to 242%, 304% and 867% of the control level, respectively, and

Table 1. The effects of six inducers and malotilate on the contents of cytochromes P-450 and b_5 and the activity of NADPH-cytochrome *c* reductase

	P-450 (nmol/mg MS prot.)	b_5 (nmol/mg MS prot.)	NADPH-cyt. <i>c</i> reductase (nmol/mg MS prot./min)
Control (n=6)	1.12±0.11 (100%)	0.27±0.04 (100%)	102.12±17.18 (100%)
PB (n=6)	2.10±0.32* (188%)	0.49±0.04* (181%)	163.22± 8.22* (160%)
3-MC (n=6)	1.74±0.14* (155%)	0.47±0.04* (174%)	88.25± 7.22 (86.4%)
β -NF (n=5)	2.11±0.21* (188%)	0.53±0.04* (196%)	90.99±10.96 (89.1%)
Isosafrole (n=7)	1.79±0.10* (160%)	0.63±0.05* (233%)	273.13±14.79* (267%)
PCB (n=6)	2.30±0.13* (205%)	0.58±0.03* (215%)	112.64±12.43 (110%)
INH (n=6)	1.01±0.16 (90.2%)	0.44±0.06* (163%)	104.44±18.56 (102%)
Malotilate (n=6)	0.82±0.10* (73.2%)	0.50±0.05* (185%)	195.83±32.78* (192%)

The drugs administered were as follows: phenobarbital (PB), 100 mg/kg, i.p., for 6 days; 3-methylcholanthrene (3-MC), 40 mg/kg, i.p., for 3 days; β -naphthoflavone (β -NF), 40 mg/kg, i.p., for 3 days; isosafrole, 150 mg/kg, i.p., for 3 days; polychlorinated biphenyls (PCB), 100 mg/kg, i.p., for 3 days; isoniazid (INH), 50 mg/kg, i.p., for 10 days; malotilate, 500 mg/kg, p.o., for 3 days. Liver microsomes were prepared for the assays 24 hr after the final administration of the drugs. Each value represents the mean±S.D. *Significantly different from the control ($P < 0.01$).

Fig. 1. The effects of phenobarbital (PB), 3-methylcholanthrene (3-MC), β -naphthoflavone (β -NF), isosafrole, polychlorinated biphenyls (PCB), isoniazid (INH) and malotilate on 7-methoxycoumarin (7-MC) O-demethylase activity. Each drug was administered as follows: PB, 100 mg/kg, i.p., for 6 days; 3-MC, 40 mg/kg, i.p., for 3 days; β -NF, 40 mg/kg, i.p., for 3 days; isosafrole, 150 mg/kg, i.p., for 3 days; PCB, 100 mg/kg, i.p., for 3 days; INH, 50 mg/kg, i.p., for 10 days; malotilate, 500 mg/kg, p.o., for 3 days. Liver microsomes were prepared for the assays 24 hr after the final administration of the drugs. Vertical bars represent the mean $\pm$ S.D. *Significantly different from the control ($P < 0.01$).

the ratio of the three O-dealkylase activities was 1.00:1.36:0.45.

By the 3-MC treatment, 7-EC O-deethylase and 7-PC O-depropylase activities were markedly enhanced to 1520% and 11100% of the control level, respectively, whereas 7-MC O-demethylase activity was increased merely to 142%, and the ratio of the three O-dealkylase activities was 1.00:11.65:9.76. Likewise, the β -NF treatment markedly enhanced 7-EC O-deethylase and 7-PC O-depropylase activities to 1140% and 7700% of the control level, respectively, although 7-MC O-demethylase activity was not signifi-

cantly affected (125% of the control level). The ratio of the three O-dealkylase activities was 1.00:9.87:7.70. The isosafrole treatment also enhanced 7-EC O-deethylase and 7-PC O-depropylase activities to 923% and 6300% of the control level, respectively, whereas 7-MC O-demethylase activity was increased merely to 142%, and the ratio of the three O-dealkylase activities was 1.00:7.06:5.56.

The effects of these three compounds, 3-MC, β -NF and isosafrole, which induce so-called P-448, were similar to each other; that is, they markedly enhanced 7-EC O-deethylase and 7-PC O-depropylase activities,

Fig. 2. The effects of phenobarbital (PB), 3-methylcholanthrene (3-MC), β -naphthoflavone (β -NF), isosafrole, polychlorinated biphenyls (PCB), isoniazid (INH) and malotilate on 7-ethoxycoumarin (7-EC) O-deethylase activity. Each drug was administered as follows: PB, 100 mg/kg, i.p., for 6 days; 3-MC, 40 mg/kg, i.p., for 3 days; β -NF, 40 mg/kg, i.p., for 3 days; isosafrole, 150 mg/kg, i.p., for 3 days; PCB, 100 mg/kg, i.p., for 3 days; INH, 50 mg/kg, i.p., for 10 days; malotilate, 500 mg/kg, p.o., for 3 days. Liver microsomes were prepared for the assays 24 hr after the final administration of the drugs. Vertical bars represent the mean $\pm$ S.D. *Significantly different from the control ($P < 0.01$).

but not 7-MC O-demethylase activity. Concerning 7-MC O-demethylase activity, no significant difference was observed among the 3-MC-, β -NF- and isosafrole-treated groups. In contrast, 7-EC O-deethylase and 7-PC O-depropylase activities in these groups were significantly different from each other, so that the ratio of the three O-dealkylase activities was slightly but clearly different from each other.

The PCB treatment enhanced the O-

dealkylase activities toward 7-MC, 7-EC and 7-PC to 221%, 1040% and 7230% of the control level, respectively, and the ratio of the three O-dealkylase activities was 1.00:5.09:4.09. The 7-MC O-demethylase activity in the PCB-treated group was similar to that in the PB-treated group, and no significant difference was observed between the two groups. In contrast, 7-EC O-deethylase and 7-PC O-depropylase activities in the PCB-treated group were similar to those in the β -

Fig. 3. The effects of phenobarbital (PB), 3-methylcholanthrene (3-MC), β -naphthoflavone (β -NF), isosafrole, polychlorinated biphenyls (PCB), isoniazid (INH) and malotilate on 7-propoxycoumarin (7-PC) O-depropylase activity. Each drug was administered as follows: PB, 100 mg/kg, i.p., for 6 days; 3-MC, 40 mg/kg, i.p., for 3 days; β -NF, 40 mg/kg, i.p., for 3 days; isosafrole, 150 mg/kg, i.p., for 3 days; PCB, 100 mg/kg, i.p., for 3 days; INH, 50 mg/kg, i.p., for 10 days; malotilate, 500 mg/kg, p.o., for 3 days. Liver microsomes were prepared for the assays 24 hr after the final administration of the drugs. Vertical bars represent the mean $\pm$ S.D. *Significantly different from the control ($P < 0.01$).

NF- and isosafrole-treated groups, and no significant difference was observed between the PCB- and β -NF-treated groups and between the PCB- and isosafrole-treated groups.

In the INH-treated group, the O-dealkylase activities toward 7-MC, 7-EC and 7-PC were significantly increased to similar extents; that is, 263%, 288% and 233% of the control level, respectively. The ratio of the three O-dealkylase activities in the INH-treated group was 1.00:1.19:0.11, which was similar to that in the control group. Concerning 7-MC O-demethylase and 7-EC O-deethylase activities, no significant difference was observed

between the INH- and PB-treated groups.

On the other hand, the malotilate treatment enhanced the O-dealkylase activities toward 7-MC, 7-EC and 7-PC to 217%, 415% and 1400% of the control level, respectively. The 7-MC O-demethylase activity in the malotilate-treated group was similar to that in the PB-treated group, and no significant difference was observed between the two groups. However, the ratio of the three O-dealkylase activities in the malotilate-treated group was 1.00:2.08:0.81, which was different from that in any other inducer-treated group examined.

Table 2. A comparison of the effects of six inducers and malotilate on the O-dealkylase activities toward 7-methoxycoumarin (7-MC), 7-ethoxycoumarin (7-EC) and 7-propoxycoumarin (7-PC)

	7-MC O-demethylation	7-EC O-deethylation (nmol/nmol P-450/min)	7-PC O-depropylation
Control (n=6)	0.24±0.05 (1.00)	0.26±0.05 (1.08)	0.030±0.009 (0.13)
PB (n=6)	0.58±0.11 (1.00)	0.79±0.21 (1.36)	0.26±0.08 (0.45)
3-MC (n=6)	0.34±0.05 (1.00)	3.96±0.57 (11.65)	3.32±0.49 (9.76)
β -NF (n=5)	0.30±0.06 (1.00)	2.96±0.42 (9.87)	2.31±0.36 (7.70)
Isosafrole (n=7)	0.34±0.05 (1.00)	2.40±0.29 (7.06)	1.89±0.31 (5.56)
PCB (n=6)	0.53±0.07 (1.00)	2.70±0.38 (5.09)	2.17±0.29 (4.09)
INH (n=6)	0.63±0.11 (1.00)	0.75±0.11 (1.19)	0.070±0.021 (0.11)
Malotilate (n=6)	0.52±0.08 (1.00)	1.08±0.18 (2.08)	0.42±0.07 (0.81)

The drugs administered were as follows: phenobarbital (PB), 100 mg/kg, i.p., for 6 days; 3-methylcholanthrene (3-MC), 40 mg/kg, i.p., for 3 days; β -naphthoflavone (β -NF), 40 mg/kg, i.p., for 3 days; isosafrole, 150 mg/kg, i.p., for 3 days; polychlorinated biphenyls (PCB), 100 mg/kg, i.p., for 3 days; isoniazid (INH), 50 mg/kg, i.p., for 10 days; malotilate, 500 mg/kg, p.o., for 3 days. Liver microsomes were prepared for the assays 24 hr after the final administration of the drugs. Each value represents the mean±S.D.

Discussion

P-450, which is a key enzyme in the mixed-function oxidase system in liver microsomes, consists of multiple species; various compounds induce a different form(s) of P-450 (2, 3). Imai (3) summarized the relationship between the molecular species of P-450 in rats and the compounds which induce them. According to his review, PB mainly induces P-450b,e; 3-MC mainly induces P-450c,d; β -NF mainly induces P-450c; isosafrole mainly induces P-450d; PCB mainly induces P-450b,c,d,e; and INH mainly induces P-450j (the names of P-450 species are based on the nomenclature by Levin et al. (4-9)).

The effects of these six inducers on 7-alkoxycoumarin O-dealkylase activities were examined in this study, which confirmed that all of the O-dealkylase activities toward 7-MC, 7-EC and 7-PC were stimulated by the PB treatment; in contrast, the 3-MC and β -NF treatments markedly enhanced 7-EC O-deethylase and 7-PC O-depropylase activi-

ties, but not 7-MC O-demethylase activity, as described by Matsubara et al. (10). The isosafrole treatment also markedly enhanced 7-EC O-deethylase and 7-PC O-depropylase activities, but not 7-MC O-demethylase activity, as reported by Kamataki et al. (12). So-called P-448-inducing agents, 3-MC, β -NF and isosafrole, similarly affected 7-alkoxycoumarin O-dealkylase activities, but the ratios of 7-EC O-deethylase and 7-PC O-depropylase to 7-MC O-demethylase activity in these three inducer-treated groups were slightly but clearly different from each other. These differences might have indicated the slight differences in the molecular forms of P-450 induced by 3-MC, β -NF and isosafrole, although they all induced so-called P-448(s).

PCB induced P-450b,c,d,e, that is both PB-induced forms (P-450b,e) and the so-called P-448s (P-450c,d). The effect of PCB on 7-alkoxycoumarin O-dealkylase activities was similar to those of both PB and P-448-inducing agents. The 7-MC O-demethylase

activity in the PCB-treated group was similar to that in the PB-treated group; on the other hand, 7-EC O-deethylase and 7-PC O-depropylase activities were similar to those in the β -NF- and isosafrole-treated groups.

The effect of INH, which induced P-450_j, on 7-alkoxycoumarin O-dealkylase activities was completely different from those of PB, P-448-inducing agents and PCB. All of the O-dealkylase activities toward 7-MC, 7-EC and 7-PC were stimulated by the INH treatment to similar extents, so that the ratio of the three O-dealkylase activities was similar to the control group.

As described above, the effects of the six inducers examined, each of which induces a different form(s) of P-450, on 7-alkoxycoumarin O-dealkylase activities were different. In particular, the ratio of 7-EC O-deethylase and 7-PC O-depropylase to 7-MC O-demethylase activity was clearly different among the groups treated with the six inducers, suggesting that the ratio of these three activities were very sensitive in reflecting the molecular form(s) of P-450 induced. Therefore, the measurement of the O-dealkylase activities toward 7-MC, 7-EC and 7-PC and the determination of the ratio of these three O-dealkylase activities should be extremely useful for the routine determination of the molecular species of P-450.

On the other hand, the effect of malotilate, which is considered a new type of inducer (13, 14), on 7-alkoxycoumarin O-dealkylase activities was clearly different from that of any other inducer examined in these experiments. Therefore, malotilate may have affected the population of P-450 species, and there might be a possibility that malotilate induced a form(s) of P-450 that is different from any of the already known species. Kawata et al. (19) recently demonstrated using reconstituted systems that the stimulatory effect of b_5 on p-nitroanisole O-demethylase activity and N-demethylase activities toward aminopyrine and benzphetamine in the P-450 preparation from malotilate-treated rats was more conspicuous than that in the preparation from control rats. They suggested that the microsomes of the malotilate-treated rats contained a form(s) of P-450 which required b_5 for the maximal activities of the demethyl-

ation reactions. Their report supports our observations that malotilate might have affected the molecular species of P-450.

The measurement of 7-alkoxycoumarin O-dealkylase activities has some advantages. First, this method does not require the use of dangerous carcinogenic compounds nor organic solvents, as Matsubara et al. (10, 20) also pointed out concerning their own methods; benzo(a)pyrene hydroxylation, for example, needs organic solvents for the assay and produces carcinogenic metabolites, although it is very specific for P-448. Secondly, an individual difference was scarcely observed concerning the ratio of 7-EC O-deethylase and 7-PC O-depropylase to 7-MC O-demethylase activity, although the absolute value of these activities in each rat varied to some extent. For example, in the six rats treated with PCB, the ratios of 7-EC O-deethylase to 7-MC O-demethylase activity were 4.72, 5.03, 5.48, 4.95, 5.02 and 5.40; the ratios of 7-PC O-depropylase to 7-MC O-demethylase activity were 3.84, 4.01, 4.42, 4.02, 4.03 and 4.27; and the ratio of the three O-dealkylase activities in this group was $1.00:5.10 \pm 0.29:4.10 \pm 0.21$ (mean $\pm$ S.D.). A similar tendency was observed in the other groups (data not shown). Third, as Matsubara et al. (20, 21) already reported, these O-dealkylase activities could be measured using not only liver microsomes but also whole liver homogenates. Indeed, this method is very sensitive, so that these O-dealkylase activities can be measured using at least 10 mg of a liver specimen obtained by human liver biopsy (data not shown). Therefore, this method is clinically applicable for the determination of the drug-metabolizing activity in the human liver. Further studies along this line are in progress in our laboratory.

References

- 1 Kato, R.: Possible role of P-450 in the oxidation of drugs in liver microsomes. *J. Biochem.* **59**, 574–583 (1966)
- 2 Imai, Y. and Sato, R.: Multiplicity of hepatic microsomal cytochrome P-450: Molecular species and substrate specificity. *Taisha* **19**, 1723–1735 (1982) (in Japanese)
- 3 Imai, Y.: Molecular multiplicity and nomenclature of hepatic microsomal cytochrome P-450. *Saibokogaku* **6**, 98–108 (1987) (in Japanese)

- 4 Ryan, D.E., Thomas, P.E., Korzeniowski, D. and Levin, W.: Separation and characterization of highly purified forms of liver microsomal cytochrome P-450 from rats treated with polychlorinated biphenyls, phenobarbital, and 3-methylcholanthrene. *J. Biol. Chem.* **254**, 1365–1374 (1979)
- 5 Ryan, D.E., Thomas, P.E. and Levin, W.: Hepatic microsomal cytochrome P-450 from rats treated with isosafrole: Purification and characterization of four enzymic forms. *J. Biol. Chem.* **255**, 7941–7955 (1980)
- 6 Ryan, D.E., Thomas, P.E. and Levin, W.: Purification and characterization of a minor form of hepatic microsomal cytochrome P-450 from rats treated with polychlorinated biphenyls. *Arch. Biochem. Biophys.* **216**, 272–288 (1982)
- 7 Ryan, D.E., Iida, S., Wood, A.W., Thomas, P.E., Lieber, C.S. and Levin, W.: Characterization of three highly purified cytochromes P-450 from hepatic microsomes of adult male rats. *J. Biol. Chem.* **259**, 1239–1250 (1984)
- 8 Haniu, M., Ryan, D.E., Iida, S., Lieber, C.S., Levin, W. and Shively, J.E.: NH₂-terminal sequence analyses of four rat hepatic microsomal cytochromes P-450. *Arch. Biochem. Biophys.* **235**, 304–311 (1984)
- 9 Ryan, D.E., Ramanathan, L., Iida, S., Thomas, P.E., Haniu, M., Shively, J.E., Lieber, C.S. and Levin, W.: Characterization of a major form of rat hepatic microsomal cytochrome P-450 induced by isoniazid. *J. Biol. Chem.* **260**, 6385–6393 (1985)
- 10 Matsubara, T., Yoshihara, E., Iwata, T., Tochino, Y. and Hachino, Y.: Biotransformation of coumarin derivatives (1): 7-alkoxycoumarin O-dealkylase in liver microsomes. *Japan. J. Pharmacol.* **32**, 9–21 (1982)
- 11 Kasahara, A., Hayashi, N., Sato, N., Meren, H., Saito, M., Yoshihara, H., Fusamoto, H., Kamada, T. and Abe, H.: Study on drug-metabolizing enzyme system by coumarin derivatives. *Acta Hepatol. Japon.* **23**, 873–878 (1982) (Abs. in English)
- 12 Kamataki, T., Ando, M., Yamazoe, Y., Ishii, K. and Kato, R.: Sex difference in the O-dealkylation activity of 7-hydroxycoumarin O-alkyl derivatives in liver microsomes of rats. *Biochem. Pharmacol.* **29**, 1015–1022 (1980)
- 13 Katoh, M., Kitada, M., Satoh, T., Kitagawa, H., Sugimoto, T. and Kasai, T.: Further studies on the in vivo effect of diisopropyl 1,3-dithiol-2-ylidenemalonate (NKK-105) on the liver microsomal drug oxidation system in rats. *Biochem. Pharmacol.* **30**, 2759–2765 (1981)
- 14 Okuno, H., Murase, T., Nakanishi, S., Shiozaki, Y. and Sameshima, Y.: Effect of malotilate (diisopropyl 1,3-dithiol-2-ylidenemalonate) on drug metabolizing activity in rat liver microsomes. *Japan. J. Pharmacol.* **44**, 303–310 (1987)
- 15 Omura, T. and Sato, R.: The carbon monoxide-binding pigment of liver microsomes. *J. Biol. Chem.* **239**, 2370–2385 (1964)
- 16 Omura, T. and Takesue, S.: A new method for simultaneous purification of cytochrome *b₅* and NADPH-cytochrome *c* reductase from rat liver microsomes. *J. Biochem.* **67**, 249–257 (1970)
- 17 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275 (1951)
- 18 Ullrich, V. and Weber, P.: The O-dealkylation of 7-ethoxycoumarin by liver microsomes: A direct fluorometric test. *Hoppe Seylers Z. Physiol. Chem.* **353**, 1171–1177 (1972)
- 19 Kawata, S., Sugiyama, T., Seki, K., Tarui, S., Okamoto, M. and Yamano, T.: Stimulatory effect of cytochrome *b₅* induced by p-nitroanisole and diisopropyl 1,3-dithiol-2-ylidenemalonate on rat liver microsomal drug hydroxylations. *J. Biochem.* **92**, 305–313 (1982)
- 20 Matsubara, T., Otsubo, S., Yoshihara, E. and Touchi, A.: Biotransformation of coumarin derivatives (2): Oxidative metabolism of 7-alkoxycoumarin by microsomal enzymes and a simple assay procedure for 7-alkoxycoumarin O-dealkylase. *Japan. J. Pharmacol.* **33**, 41–56 (1983)
- 21 Matsubara, T., Yamada, N., Mitomi, T., Yokoyama, S., Fukiishi, Y., Hasegawa, Y. and Nishimura, H.: Cytochrome P-450-dependent monooxygenase activities in prenatal and postnatal human livers: Comparison of human liver 7-alkoxycoumarin O-dealkylases with rat liver enzymes. *Japan. J. Pharmacol.* **40**, 389–398 (1986)