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Lead Nitrate Inhibits the Induction of CYP1A mRNAs by Aromatic Amines but not by Aryl Hydrocarbons in the Rat Liver[1]

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The effects of lead nitrate on the induction of hepatic cytochrome P450IA (CYP1A) isoforms, mainly CYP1A2, by aromatic amines (2-methoxy-4-aminoazobenzene, 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine) and aryl hydrocarbons (3-methylcholanthrene, benzo[*a*]pyrene and β-naphthoflavone) in male F344 rats were examined at the levels of mRNA, protein and activity of the enzymes. Pretreatment of rats with lead nitrate suppressed the expression of hepatic CYP1A enzyme(s), especially CYP1A2, at both levels of protein and activity of the enzyme(s) by treatment with an aromatic amine or an aryl hydrocarbon. On the other hand, the lead nitrate pretreatment suppressed the induction of CYP1A mRNA(s) by an aromatic amine but not by an aryl hydrocarbon. These findings indicate that lead nitrate suppresses the expression of CYP1A enzymes at both stages of post-translation of mRNAs and transcriptional activation of the genes, and further suggest that the pathway for the transcriptional activation of the *CYP1A* genes by the aromatic amines is different from that by the aryl hydrocarbons.

Key words: aromatic amine, aryl hydrocarbon, CYP1A, cytochrome P450, lead nitrate.

Administration of lead nitrate to rats induces the placental form of glutathione *S*-transferase (*1*), a marker enzyme for (pre)neoplasitic lesion in the rat liver (*2*), but decreases the total amount of microsomal cytochrome P450 (CYP) enzymes, especially CYP1A2, in the liver (*3-5*). The changes in hepatic enzymes induced by lead nitrate are similar to those in liver hyperplastic nodules of the rat (*5-7*). The decrease in the level of CYP enzymes including CYP1A2 might be attributable to the decrease in the level of heme, an essential component of CYP enzyme, because lead nitrate, like other ionic metals such as cadmiun, cobalt, and nickel, induces heme oxygenase (*8-10*). However, of these metal ions, only lead ion showed an inhibitory effect on the expression of CYP1A2 enzyme at the level of the mRNA (*3, 4*), suggesting that lead nitrate suppresses the expression of CYP1A2 enzyme at both stages of post-translation of mRNA and transcription of the gene.

Since CYP1A2 is induced concomitantly with CYP1A1 in a CYP1A inducer-treated rat liver, induction of the CYP1A enzymes is thought to occur through the same pathway. However, although the induction of CYP1A enzyme(s), especially CYP1A1, by an aryl hydrocarbon (Ah) is initiated by formation of Ah-cytosolic Ah receptor complex (*11-13*), some of the aromatic amine-type CYP1A inducers have little affinity for the Ah receptor (*14*). Furthermore, the aromatic amine-type CYP1A inducers, such as 4-aminoazobenzene derivatives (*15, 16*) and carcinogenic heterocyclic amines (*17*), induce predominantly CYP1A2 rather than CYP1A1, as opposed to the aryl hydrocarbon type-inducers, such as 3-methylcholanthrene (MC), benzo[*a*]pyrene (B[*a*]P) and β-naphthoflavone (β-NF), which predominantly induce CYP1A1 (*18-20*). The differences in biochemical properties between the aromatic amines and the aryl hydrocarbons suggest that these chemicals induce CYP1A enzymes *via* different pathways.

In this study, to understand the pathway of the expression of CYP1A enzymes, we examined the effects of lead nitrate, an inhibitor of the expression of CYP1A enzymes (*3-5*), on the induction of CYP1A enzymes in the rat liver by different types of CYP1A inducers (aromatic amines, CYP1A2-selective inducers; aryl hydrocarbons, CYP1A1-selective inducers). We report herein that lead nitrate inhibited the induction of CYP1A mRNA(s) by aromatic amines but not by aryl hydrocarbons and suggest that at least two pathways operate for the transcriptional activation of the *CYP1A* gene(s).

MATERIALS AND METHODS

Chemicals—2-Amino-6-methyl-dipyrido[1,2-*a*;3′,2′-*d*]-imidazole hydrochloride (Glu-P-1), 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole acetate (MeAαC) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) were kindly donated by Drs. T. Sugimura and M. Nagao, National Cancer Center Research Institute, Tokyo. Lead nitrate, 3-

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Abbreviations: CYP, cytochrome P450; MC, 3-methylcholanthrene; B[*a*]P, benzo[*a*]pyrene; β-NF, β-naphthoflavone; Glu-P-1, 2-amino-6-methyl-dipyrido[1,2-*a*;3′,2′-*d*]imidazole hydrochloride; MeAαC, 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole acetate; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; 2-MeO-AAB, 2-methoxy-4-aminoazobenzene.

methylcholanthrene (MC), benzo[a]pyrene (B[a]P), and β-naphthoflavone (β-NF) were purchased from Wako Pure Chemical Industries, Osaka. 2-Methoxy-4-aminoazobenzene (2-MeO-AAB) was synthesized in our laboratory by the method described previously (*16*).

Treatments of Rats with Chemicals—Male F344 rats were purchased from Japan SLC, Hamamatsu, and used at 7 weeks of age. They were kept in plastic cages in an air-conditioned room. All rats were given a CE-2 diet (CLEA Japan, Tokyo) and water *ad libitum*. Some of rats were given an i.v. injection of 0.5 ml of lead nitrate (100 μmol/kg) dissolved in distilled water, followed 9 h later by an i.p. injection of an aromatic amine (2-MeO-AAB, MeAαC or PhIP; 0.22 mmol/kg), or an aryl hydrocarbon (MC, B[a]P or β-NF; 0.11 mmol/kg) in corn oil. Other rats were treated with a CYP1A inducer (aromatic amine or aryl hydrocarbon) alone. All rats were killed 15 h after treatment with a CYP1A inducer. The liver from individual rats was cut into several blocks and stored at −80°C until use.

Preparation of Liver Microsomes—The liver was homogenized with 3 volumes (v/w) of 1.15% KCl using a Polytron homogenizer (Kinematica, Switzerland). Microsomal pellets were prepared from the homogenates by differential centrifugations as described previously (*17*) and resuspended in 1.15% KCl. The amounts of protein and CYP in microsomal fractions were assayed by the methods of Lowry *et al.* (*21*) and Omura and Sato (*22*), respectively.

Mutation Test—Microsomal activity for mediating mutagenic activation of Glu-P-1 (2 nmol/plate) was assayed by mean of Ames' bacterial mutation test using *Salmonella typhimurium* TA98 as a tester strain, as described previously (*17*). Throughout the present experiment, the number of spontaneous revertant colonies (without a substrate) was in the range of 10-40. Data shown are the values obtained by subtracting the number of spontaneous revertant colonies from total number of colonies.

Western Blot Analysis of CYP1A Proteins—Western blot analysis of CYP1A proteins was performed as described previously (*17*). In brief, microsomal preparations were solubilized with sodium dodecyl sulfate (SDS) and developed by SDS-PAGE. The separated proteins were transferred from the gel to a nitrocellulose sheet, then immunostained by means of protein A-enzyme linked immunosorbent assay (protein A-ELISA) using an anti-CYP1A1 monoclonal antibody, APL-2, which is cross-reactive with CYP1A2 (*17*), and 0.05% 3,3′-diaminobenzidine tetrachloride (Sigma).

Northern Blot Analysis of CYP1A mRNAs—Total cellular RNAs of liver tissues were isolated by the method of Chomczynski and Sacchi (*23*). RNA preparations were denatured in 20 mM 3-(*N*-morpholino)propane sulfonate buffer, pH 7.0, containing 2.2 M formaldehyde, 15 mM sodium acetate, 1 mM EDTA, and 50% formamide, for 15 min at 60°C. Equivalence of loading was checked by ethidium bromide staining of 28S and 18S rRNAs on a formaldehyde-agarose gel. CYP1A mRNAs were assayed as described previously (*24*) using oligonucleotide probes, 5′-GAGATGCTGAGGACCAGAAGACCG-3′ for CYP1A1 mRNA and 5′-TTCACTT-GGAGAAGCGTGGCCAGGCC-3′ for CYP1A2 mRNA. The band(s) corresponding to CYP1A mRNA(s) on a sheet was quantitated with Bio-Rad Molecular Imager GS-250, then sheets were submitted to autoradiography at −80°C for 48 h with X-OMAT film (Kodak).

TABLE I. **Effects of lead nitrate on the amount of total CYP enzymes in hepatic microsomes and on the microsomal activity for Glu-P-1 mutagenesis.**

CYP inducer	Lead nitrate pretreatment[a]	CYP content (nmol/mg protein)	Microsomal activity for Glu-P-1 mutagenesis (revertant colonies/mg protein)
None	−	0.54±0.04	900±140
None	+	0.25±0.05[b]	430±10[c]
2-MeO-AAB	−	0.46±0.03	8,100±1,200
2-MeO-AAB	+	0.27±0.01[c]	2,700±160[c]
MeAαC	−	0.50±0.02	5,300±480
MeAαC	+	0.26±0.01[c]	2,300±240[c]
PhIP	−	0.63±0.04	1,300±120
PhIP	+	0.23±0.02[b]	310±20[b]
MC	−	0.56±0.02	17,300±800
MC	+	0.32±0.03[c]	8,200±600[c]
B[a]P	−	0.52±0.02	15,300±900
B[a]P	+	0.29±0.03[d]	7,100±380[c]
β-NF	−	0.62±0.03	16,000±600
β-NF	+	0.44±0.03[d]	8,300±300[c]

The assays were performed using microsomes prepared from the pooled livers of three rats in each experimental group. All data represent the mean±SE of triplicate samples. [a] −, without lead nitrate; +, with lead nitrate. [b-d] Significantly different from the corresponding controls (assayed by Student's *t*-test): [b] $p<0.01$; [c] $p<0.001$; [d] $p<0.05$.

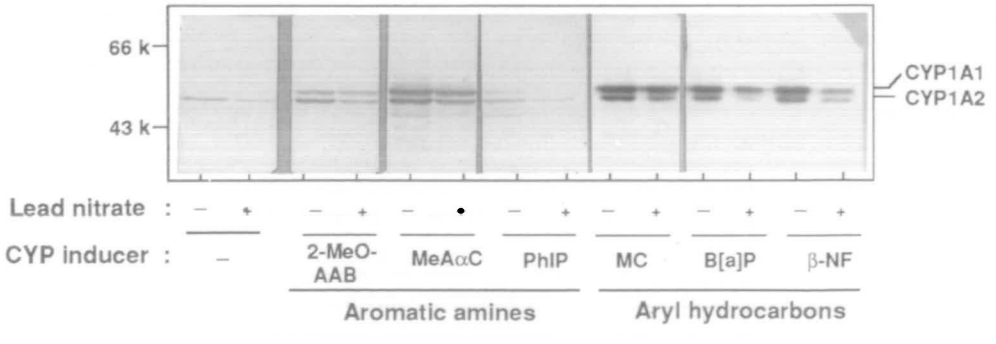

Fig. 1. **Western blot analysis of microsomal CYP1A proteins in the liver of rats treated with a combination of lead nitrate and an aromatic amine or an aryl hydrocarbon.** Microsomes were prepared from the pooled livers of three untreated rats (control) or three rats treated with lead nitrate and/or a CYP1A inducer, as described in "MATERIALS AND METHODS." The microsomal preparation [60 μg protein/lane for a CYP1A inducer-untreated (control) rats; 40 μg/lane for 2-MeO-AAB-, MeAαC-, or PhIP-treated rats; 10 μg/lane for MC, B[a]P, and β-NF-treated rats] was subjected to Western blot analysis using an APL-2 MoAb reactive with both CYP1A proteins, as described in "MATERIALS AND METHODS." K, kilodalton. The experiments were repeated twice with similar results.

RESULTS

We have previously reported that pretreatment of rats with lead nitrate suppressed the induction of CYP1A2 by 2-MeO-AAB at the levels of mRNA, protein and activity, whereas simultaneous or post-treatment showed little suppressive effect on the CYP1A2 induction (*4*). Therefore, lead nitrate was given to male F344 rats 9 h before treatment with a CYP1A inducer: an aromatic amine, 2-MeO-AAB (*16*), MeAαC (*17*), or PhIP (*25*); or an aryl hydrocarbon, MC, B[*a*]P or β-NF (*18-20*). Rats were killed 15 h after treatment with the CYP1A inducer.

The total amount of CYPs in hepatic microsomes was not significantly changed by treatment with any of the CYP1A inducers (Table I). Pretreatment of rats with lead nitrate resulted in a significant decrease in the total amount of CYPs, as compared to the corresponding controls. Microsomal activity was assessed by means of a bacterial mutation test using Glu-P-1 as a substrate for CYP1A enzymes, mainly CYP1A2 (*26*). Treatment of rats with an aromatic amine (2-MeO-AAB, MeAαC, or PhIP) or an aryl hydrocarbon (MC, B[*a*]P, or β-NF) resulted in a significant increase of the microsomal activity for the bioactivation of Glu-P-1 (Table I). Increased levels of the microsomal activity in CYP1A inducer-treated rats were significantly reduced by pretreatment with lead nitrate.

Representative profiles of Western blots of microsomal CYP1A proteins are shown in Fig. 1. Treatment of rats with an aromatic amine (2-MeO-AAB, MeAαC, or PhIP) or an aryl hydrocarbon (MC, B[*a*]P, or β-NF) resulted in an increase in the levels of CYP1A proteins (Table II). In all cases, the increased levels of CYP1A proteins, especially CYP1A2 protein, were suppressed by pretreatment with lead nitrate.

The effects of lead nitrate on the level of the CYP1A mRNAs induced by the treatment with an aromatic amine or an aryl hydrocarbon were further examined by Northern blotting. The level of CYP1A2 mRNA was increased by treatment with either type of inducer (Fig. 2). Pretreatment with lead nitrate suppressed the induction of CYP1A2 mRNA by the aromatic amines but not by the aryl hydrocarbons. CYP1A1 mRNA was undetectable in CYP1A inducer-untreated rats and in rats treated with an aromatic amine (2-MeO-AAB or PhIP) (Fig. 3). Treatment with MeAαC or an aryl hydrocarbon (MC, B[*a*]P, or β-NF) resulted in an increase in the level of CYP1A1 mRNA in the rat liver. Pretreatment with lead nitrate suppressed the induction of CYP1A1 mRNA by MeAαC but not by the aryl hydrocarbon.

TABLE II. **Suppressive effect of lead nitrate pretreatment on the expression of CYP1A proteins in rat liver.**

Lead nitrate-pretreatment[a]	CYP inducer-treatment	Amount of CYP1A proteins (arbitrary units/60 μg protein) CYP1A1	CYP1A2
−	none	n.d[b]	0.06
+	none	n.d	0.02 (33)
−	2-MeO-AAB	0.18	0.29
+	2-MeO-AAB	0.11 (57)[c]	0.14 (48)
−	MeAαC	0.50	0.60
+	MeAαC	0.32 (64)	0.28 (47)
−	PhIP	0.04	0.12
+	PhIP	0.01 (25)	0.02 (17)
−	MC	3.00	1.92
+	MC	1.91 (64)	0.86 (45)
−	B[*a*]P	2.77	1.07
+	B[*a*]P	1.88 (67)	0.39 (36)
−	β-NF	2.88	1.54
+	β-NF	1.64 (57)	0.66 (43)

Western blot analyses of microsomal CYP1A proteins were performed as described in the legend of Fig. 1, and the amounts of separated CYP1A proteins on a nitrocellulose sheet were measured with a scanning densitometer (Densito-Pattern Analyzer EPA-3000; Maruzen Petrochemical, Japan). The data shown represent the mean of duplicate determinations. [a]−, without lead nitrate; +, with lead nitrate. [b]n.d; not detected. [c]Values in parentheses indicate per cent of the corresponding controls (lead nitrate-untreated rats).

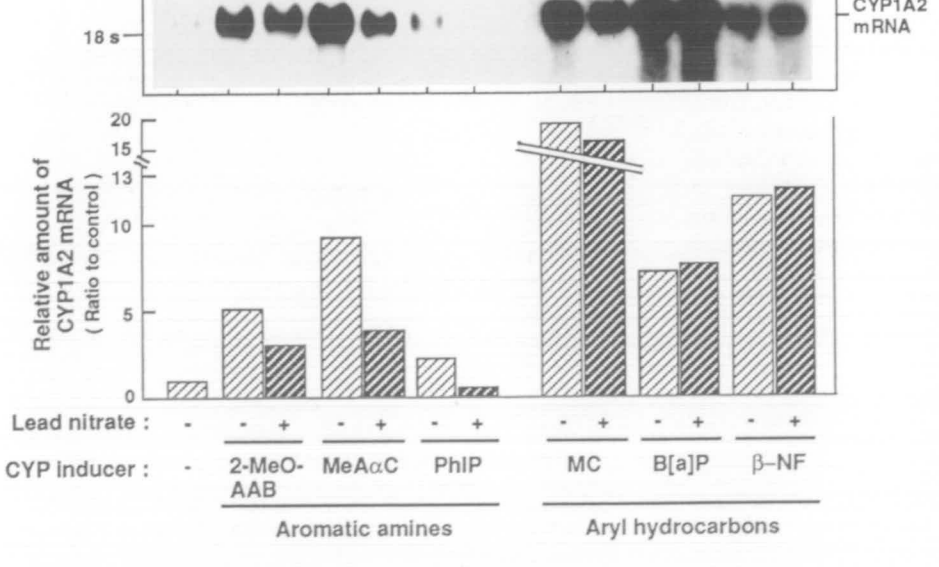

Fig. 2. **Northern blot analysis of CYP1A2 mRNA in the liver of rats treated with a combination of lead nitrate and an aromatic amine or an aryl hydrocarbon.** Total cellular RNA preparations were obtained from the pooled livers of three rats (control) untreated with chemicals (lead nitrate and CYP inducer) or three rats treated with lead nitrate and/or a CYP1A inducer, as described in "MATERIALS AND METHODS." The RNA preparation (20 μg RNA/lane for the control rats or aromatic amine-treated rats; 10 μg/lane for MC- or β-NF-treated rats; 20 μg/lane for B[*a*]P-treated rats) was subjected to Northern blot analysis using a synthetic oligonucleotide probe for CYP1A2 mRNA as described in "MATERIALS AND METHODS." Amounts of CYP1A2 mRNA (per 10 μg total RNA) in experimetal groups were compared with that of control rats. The experiments were repeated twice with similar results.

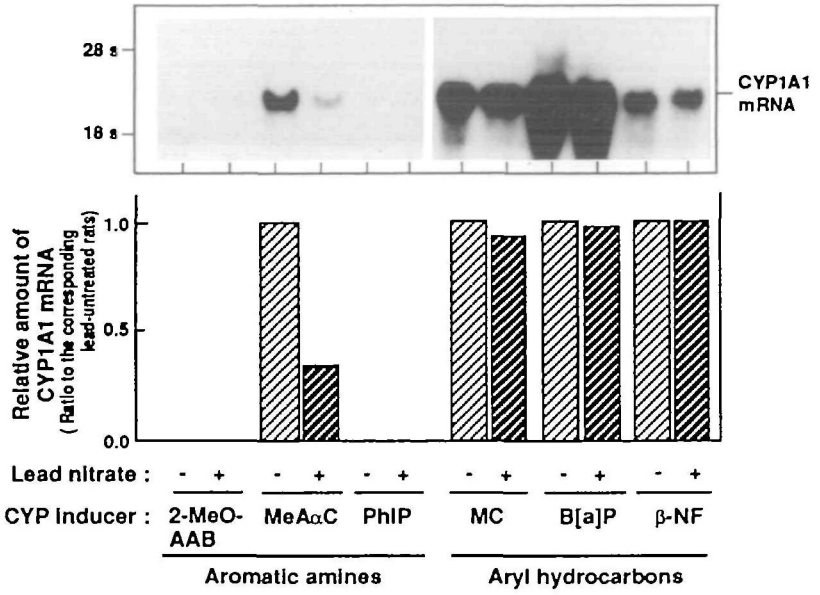

Fig. 3. **Northern blot analysis of CYP1A1 mRNA in the liver of rats treated with a combination of lead nitrate and an aromatic amine or an aryl hydrocarbon.** The assays were performed as described in the legend of Fig. 2 using a synthetic oligonucletide probe for CYP1A1 mRNA. The experiments were repeated twice with similar results.

DISCUSSION

We have previously reported that lead nitrate inhibited the induction by 2-MeO-AAB or MC of CYP1A enzymes, especially CYP1A2, at the levels of protein and activity of the enzymes (*3-5*). This was confirmed in the present experiment. The lead nitrate-mediated decrease in amount of total CYPs including CYP1A enzymes might be attributable mainly to a decrease in the level of heme, an essential component of CYP, because lead nitrate, like other heavy metal salts, induces the heme oxygenase responsible for degradation of heme (*8-10*). The level of CYP1A2 enzyme induced by a CYP1A inducer, especially an aryl hydrocarbon, was preferentially decreased as compared with that of CYP1A1 enzyme by pretreatment with lead nitrate. This would be explained by the fact that CYP1A2 enzyme is less stable than CYP1A1 enzyme (*27*).

Recently, we (*4*) have found that induction of CYP1A2 mRNA by 2-MeO-AAB in the rat liver was suppressed efficiently by pretreatment with lead nitrate but only slightly by simultaneous or post treatment, suggesting that the decrease in the level of CYP1A2 mRNA occurs after the lead nitrate-mediated decrease(s) in the expression and/or activity of the cellular factor(s) responsible for the transcriptional activation of the *CYP1A* gene(s) by the aromatic amine. Many studies using an aryl hydrocarbon as a CYP1A inducer indicate that synthesis of CYP1A mRNA(s) is initiated by the formation of a CYP1A inducer-Ah receptor complex (*11-13*). However, in the present study, we found that lead nitrate selectively inhibited the induction of CYP1A mRNA(s) by the aromatic amines but not by the aryl hydrocarbons, suggesting that cellular factor(s) other than Ah receptor play an important role in the transcriptional activation of the *CYP1A* gene(s) by the aromatic amines. This is supported by the following evidence: (i) aromatic amines such as tryptophan-pyrolysate components can induce CYP1A(s) in DBA/2 mice nonresponsive to the aryl hydrocarbon (*28*); (ii) some heterocyclic aromatic amines have little affinity for Ah receptor but show definite activity for inducing CYP1A(s) in the rat liver (*14*); (iii) 2-methoxy-4-nitroaniline is a selective CYP1A2 inducer (*29*), but the molecule lacks the structural unit requisite for its binding to Ah receptor (*30*); and (iv) acenaphthylene and its related tricyclic hydrocarbons selectively induce CYP1A2 mRNA *via* an Ah receptor-independent pathway in B6C3F1 mice (*31*).

Lead nitrate has been reported to be able to enhance the production of tumor necrosis factor-α (*32*), which decreases the constitutive level of CYP1A2 mRNA in primary-cultured human hepatocytes (*33*). These findings suggest that the decrease in the constitutive (*3*) and aromatic amine-induced levels of CYP1A2 mRNA in the rat liver caused by lead nitrate may occur, at least in part, through a TNF-α-associated mechanism.

In this study we demonstrated that lead nitrate showed different effects on the induction of CYP1A mRNA(s) by different types of CYP1A inducers, namely, aromatic amines and aryl hydrocarbons. This suggests that pathways for transcriptional activation of the *CYP1A* gene(s) by aromatic amines and aryl hydrocarbons are different from each other.

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REFERENCES

1. Roomi, M.W., Columbano, A., Ledda-Columbano, G.M., and Sarma, D.S.R. (1986) Lead nitrate induces certain biochemical properties characteristic of hepatocyte nodules. *Carcinogenesis* **7**, 1643-1646
2. Sato, K. (1989) Glutathione transferases as markers of preneoplasia and neoplasia. *Adv. Cancer Res.* **52**, 205-255
3. Degawa, M., Arai, H., Kubota, M., and Hashimoto, Y. (1994) Ionic lead, a unique metal ion as an inhibitor for cytochrome P450IA2 (CYP1A2) expression in the rat liver. *Biochem. Biophys. Res. Commun.* **200**, 1086-1092
4. Degawa, M., Arai, H., Kubota, M., and Hashimoto, Y. (1995) Ionic lead, but not other ionic metals (Ni^{2+}, Co^{2+} and Cd^{2+}), suppresses on the 2-methoxy-4-aminoazobenzene-mediated

cytochrome P450IA2 (CYP1A2) in rat liver. *Biol. Pharm. Bull.* **18**, 1215-1218
5. Degawa, M., Arai, H., Miura, S., and Hashimoto, Y. (1993) Preferential inhibitions of hepatic P450IA2 expression and induction by lead nitrate in the rat. *Carcinogenesis* **14**, 1091-1094
6. Degawa, M., Miura, S., and Hashimoto, Y. (1991) Expression and induction of cytochrome P450 isozymes in hyperplastic nodules of rat liver. *Carcinogenesis* **12**, 2151-2156
7. Buchmann, A., Schwarz, M., Schmitt, R., Wolf, C.R., Oesch, F., and Kunz, W. (1987) Development of cytochrome P-450-altered preneoplastic and neoplastic lesions during nitrosamine-induced hepatocarcinogenesis in the rats. *Cancer Res.* **47**, 2911-2918
8. Alvares, A.P., Leigh, S., Cohn, J., and Kappas, J. (1972) Lead and methylmercury: Effects of acute exposure on cytochrome P-450 and the mixed function oxidase system in the liver. *J. Exp. Med.* **135**, 1406-1409
9. Scoppa, P., Roumengous, M., and Penning, M.W. (1973) Hepatic drug metabolizing activity in lead-poisoned rats. *Experimentia* **29**, 970-972
10. Maines, M.D. and Kappas, A. (1976) Studies on the mechanism of induction of haem oxygenase by cobalt and other metal ions. *Biochem. J.* **154**, 125-131
11. Nebert, D.W. and Gonzalez, F.J. (1987) P450 gene: Structure, evolution, and regulation. *Annu. Rev. Biochem.* **56**, 945-993
12. Whitlock, J.P., Jr. (1986) The regulation of cytochrome P-450 gene expression. *Annu. Rev. Pharmacol. Toxicol.* **26**, 333-369
13. Okey, A.B. (1990) Enzyme induction in the cytochrome P-450 system. *Pharmacol. Ther.* **45**, 241-289
14. Kleman, M., Övervik, E., Mason, G., and Gustafsson, J.-Å. (1990) Effects of the food mutagens MeIQx and PhIP on the expression of cytochrome P450IA proteins in various tissues of male and female rats. *Carcinogenesis* **11**, 2185-2189
15. Degawa, M., Kojima, M., Sato, Y., and Hashimoto, Y. (1986) Induction of a high spin form of microsomal P-448 in rat liver by 4-aminoazobenzene derivatives. *Biochem. Pharmacol.* **35**, 3565-3570
16. Degawa, M., Yamada, H., Hishinuma, T., Masuko, T., and Hashimoto, Y. (1987) Organ selective induction of cytochrome P-448 isozymes in the rat by 2-methoxy-4-aminoazobenzene and 3-methylcholanthrene. *J. Biochem.* **101**, 1437-1445
17. Degawa, M., Tanimura, S., Agatsuma, T., and Hashimoto, Y. (1989) Hepatocarcinogenic heterocyclic aromatic amines that induce cytochrome P-448 isozymes, mainly cytochrome P-448H (P450IA2), responsible for mutagenic activation of the carcinogens in rat liver. *Carcinogenesis* **10**, 1119-1122
18. Thomas, P.E., Reik, L.M., Ryan, D.E., and Levin, W. (1983) Induction of two immunochemically related rat liver cytochrome P-450 isozymes, cytochromes P-450c and P-450d, by structurally diverse xenobiotics. *J. Biol. Chem.* **258**, 4590-4598
19. Åtröm, A. and DePierre, J.W. (1986) Rat liver microsomal cytochrome P-450: Purification, characterization, multiplicity and induction. *Biochim. Biophys. Acta* **853**, 1-27
20. Gonzalez, F.J. (1989) The molecular biology of cytochrome P450s. *Pharmacol. Rev.* **40**, 243-288
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. Omura, T. and Sato, R. (1964) The carbon monooxide binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. Biol. Chem.* **239**, 2370-2378
23. Chomczynski, P. and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**, 156-159
24. Degawa, M., Miura, S., Yoshinari, K., and Hashimoto, Y. (1995) Altered expression of hepatic CYP1A enzymes in rat hepatocarcinogenesis. *Jpn. J. Cancer Res.* **86**, 535-539
25. Degawa, M., Kobayashi, K., Miura, S., Arai, H., Esumi, H., Sugimura, T., and Hashimoto, Y. (1992) Species difference among experimental rodents in induction of P450IA family enzymes by 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine. *Jpn. J. Cancer Res.* **83**, 1047-1051
26. Degawa, M., Ueno, H., Miura, S., Ohta, A., and Namiki, M. (1988) A simple method for assessment of rat cytochrome P-448 isozymes responsible for the mutagenic activation of carcinogenic chemicals. *Mutat. Res.* **203**, 333-338
27. Steward, A.R., Wrighton, S.A., Pasco, D.S., Fagan, J.B., Li, D., and Guzelian, P.S. (1985) Synthesis and degradation of 3-methylcholanthrene-inducible cytochrome P-450 and their mRNAs in primary monolayer cultures of adult rat hepatocytes. *Arch. Biochem. Biophys.* **241**, 494-508
28. Degawa, M., Yamaya, C., and Hashimoto, Y. (1988) Hepatic cytochrome P-450 isozyme(s) induced by dietary carcinogenic aromatic amines preferentially in female mice of DBA/2 and other strains. *Carcinogenesis* **9**, 567-571
29. Degawa, M., Nakayama, M., Yoshinari, K., and Hashimoto, Y. (1995) 2-Methoxy-4-nitroaniline is a selective inducer of cytochrome P450IA2 (CYP1A2) in rat liver. *Cancer Lett.* **96**, 95-98
30. Runnug, P., Sjögren, M., Rannung, A., Gillner, M., Tofgrad, R., Gustafsson, J.Å., Rosenkranz, H., and Klopman, G. (1991) Use of artificial intelligence in structure-affinity correlations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) receptor ligands. *Carcinogenesis* **12**, 2007-2015
31. Chaloupka, K., Santostefano, M., Goldfarb, I.S., Liu, G., Myers, M.J., Tsyrolv, I.B., Gelboin, H.V., Krishman, V., and Safe, S. (1994) Aryl hydrocarbon (Ah) receptor-independent induction of Cyp1a2 gene expression by acenaphthylene and related compounds in B6C3F1 mice. *Carcinogenesis* **15**, 2835-2840
32. Shinozuka, H., Kubo, Y., Katyal, S.L., Coni, P., Ledda-Columbano, G.M., Columbano, A., and Nakamura, T. (1994) Roles of growth factors and tumor necrosis-α on liver cell proliferation induced in rats by lead nitrate. *Lab. Invest.* **71**, 35-41
33. Abdel-Razzak, Z., Loyer, P., Fautrel, A., Gautier, J.-C., Corcos, L., Turlin, B., Beaune, P., and Guillouzo, A. (1993) Cytokines down-regulate expression of major cytochrome P-450 enzymes in adult human hepatocytes in primary culture. *Mol. Pharmacol.* **44**, 707-715