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Apolipoprotein A-I gene expression is upregulated by polychlorinated biphenyls in rat liver

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Xenobiotics such as polychlorinated biphenyls (PCB) increase serum cholesterol level (especially high density lipoprotein cholesterol) and apolipoprotein A-I (apo A-I) level in rats. The effect of PCB on serum apo A-I and hepatic apo A-I gene expression and the relationship between apo A-I and drug-metabolizing enzymes in rats were investigated. Serum levels of cholesterol and apo A-I were increased by dietary addition of PCB in a dose-dependent manner (0–500 mg/kg diet). Hepatic apo A-I mRNA level was also elevated by PCB in a similar fashion. Serum level of cholesterol gradually increased during feeding period of PCB (200 mg/kg diet, 105 days) and reached a two-fold higher level in PCB group than in controls. The levels of serum apo A-I and hepatic apo A-I mRNA linearly elevated during feeding period of PCB and were increased 3- or 4-fold, respectively, compared to controls. Although acute administration (16 hr) of PCB, 3-methylcholanthrene, and phenobarbital induced cytochrome P-450 gene expression in the liver, hepatic apo A-I gene expression was not increased by these xenobiotics. These results indicated that the serum levels of cholesterol and apo A-I had positive correlation with hepatic level of apo A-I mRNA in rats fed PCB, and that hepatic apo A-I gene expression was dependent upon intake of PCB but was not directly related to the induction of drug-metabolizing enzymes. This study demonstrated that xenobiotic-induced hyper-alpha-cholesterolemia would be caused by the increased apo A-I gene expression and cholesterol synthesis in the liver, coordinately. (J. Nutr. Biochem. 11:568–573, 2000)

Keywords: xenobiotics; polychlorinated biphenyls; cholesterol; high density lipoprotein; apolipoprotein A-I; cytochrome P-450

Introduction

Xenobiotics such as polychlorinated biphenyls (PCB; e.g., 1,1,1-trichloro-2,2-bis[*p*-chlorophenyl]ethane; DDT) are widely distributed in the environment as harmful contaminants. We occasionally take 2,6-di-tert-2,2-butyl-*p*-cresol (BHT) and barbital derivatives as a food additive and as a drug, respectively. The administration of xenobiotics to animals causes many metabolic and pathologic changes:[1] (1) induction of hepatic drug-metabolizing enzymes,[1] (2) elevation of the serum levels of high density lipoprotein cholesterol (HDL-C) and of apolipoprotein A-I (apo A-I),[2–6] (3) appearance of cholesterol-rich very low density lipoproteins (VLDL),[6] (4) accumulation of liver lipids,[6–9] (5) enhanced lipid peroxidation,[10] (6) increased tissue and urinary ascorbic acid in rats,[11] and (7) decreased hepatic vitamin A level.[9]

The mechanism for the induction of microsomal cytochrome P-450 (CYP) by xenobiotics has been extensively investigated. Some chemicals with a chemical structure similar to that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin induce 3-methylcholanthrene (MC)-type CYP (CYP1A1 and 1A2) through the cytosolic receptor (*Ah* receptor).[12–14] However, the mechanism of the induction of CYP2B1 and 2B2 (Cyp2b9 and 2b10 in mice) by phenobarbital (PB) and many other drugs is not fully understood at present.[15–18] PCB induces both major types of drug-inducible CYPs strongly. It is thought that exposure to those xenobiotics that induce the mixed-function oxidase system is a factor in the

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Table 1 Effect of dietary levels of polychlorinated biphenyls (PCB; mg/kg diet) on body weight gain and liver weight in rats (Experiment 1)

	PCB				
	0	100	200	300	500
Body weight gain (g/14 days)	64 ± 3	57 ± 4	59 ± 3	62 ± 4	55 ± 5
Liver weight (g/100 g body weight)	4.71 ± 0.14*,a	5.60 ± 0.13[a]	7.01 ± 0.09[b]	8.29 ± 0.25[c]	8.71 ± 0.35[d]

Values are the mean ± SEM for 4 rats.
*The statistical significance of differences among values was analyzed by one-way analysis of variance.
When the result of the treatment was significant, Duncan's multiple-range test was performed.
Means within a line not followed by the same letter are significantly different ($P < 0.05$).

increase of HDL.[5] Although several enzymes related to lipid metabolism were changed by xenobiotics,[19–22] the mechanism for the elevation of serum level of total and HDL-C by feeding xenobiotics is not known. Several xenobiotics have increased the hepatic synthesis of cholesterol,[20–22] and it has been suggested that the increase in serum cholesterol due to PCB is mainly attributable to the stimulation of hepatic cholesterol synthesis.[20,21] It has also been demonstrated that feeding PCB resulted in an increase of apo A-I-rich HDL in rats.[4,5]

The apolipoproteins are important for lipoprotein metabolism as the structural components of lipoproteins, the cofactor for enzymatic reactions, and the ligand for lipoprotein receptors. Possible changes in the apolipoprotein concentration and distribution may contribute to the hypercholesterolemia induced by xenobiotics. PCB-increased serum level of apo A-I, which is a major apolipoprotein in HDL, is likely responsible for the hyper-alpha-lipoproteinemia.

In the present study, we examined the relationship between serum level of apo A-I and hepatic apo A-I gene expression. To determine the relevance of apo A-I gene expression to induction of CYP, the expression of major drug-inducible CYP species, CYP1A1/1A2 and CYP2B1/2B2, was compared to the induction of apo A-I gene expression. Here, we demonstrated that PCB-induced hyper-alpha-cholesterolemia would be caused by not only the increased cholesterol biosynthesis but also the increased apo A-I gene expression in the liver.

Materials and methods

Animals and diets

Male rats of the Wistar strain (Japan SLC, Hamamatsu, Japan), 5–6 weeks of age, and weighing about 90 g were used for these experiments. The rats were housed individually and transferred to a semipurified diet (control diet, 250 g/kg diet of casein)[4] after being fed on a commercial nonpurified diet (CE-2, Japan Clea Co., Tokyo, Japan) for 3 days. The room temperature was kept at 24°C with a 12-hr light/dark cycle (lights on from 8:00 AM to 8:00 PM). In Experiment 1, rats were fed for 2 weeks the test diets, which added 0, 100, 200, 300, or 500 mg PCB (Aroclor 1248, Mitsubishi Monsanto Co., Tokyo, Japan) per kg diet to the control diet. In Experiment 2, rats were fed the test diet containing 200 mg/kg diet of PCB for 1, 3, 7, 14, 60, and 105 days. In Experiment 3, rats were treated intraperitoneally with 100 mg/kg body weight of sodium PB, 100 mg/kg body weight of PCB, or 25 mg/kg body weight of MC at 6:00 PM for 16 hr. All diets and tap water were supplied ad libitum. At the end of the experimental periods, blood from 4-hr starved rats was collected at 1:00 PM (Experiments 1 and 2) or 11:00 AM (Experiment 3) by decapitation. Serum was separated by centrifugation at 1,500 × *g* for 10 min. Liver was quickly removed, weighed, and used for RNA isolation.

RNA isolation and Northern blot analysis

RNA was extracted according to the method of Chomczynski and Sacchi.[23] Total cellular RNA was quantified spectrophotometrically at 260 nm. Fifteen micrograms of total cellular RNA was subjected to electrophoresis on 1% agarose containing 2.2 M formaldehyde. To ensure that an equal amount of RNA was loaded, RNA was visualized with ethidium bromide. In the present study, RNA was equally loaded by observation of 28S and 18S ribosomal RNA stained with ethidium bromide. RNA was transferred to Hybond-N+ membrane (Amersham, USA) by overnight capillary blotting. The membranes were baked at 80°C, then prehybridized at 42°C for at least 8 hr. The amount of *32*P-labeled cDNA probe added to the hybridization solution was 6×10^6 cpm/mL, and hybridization was carried out for 24 hr. After washing, filters were exposed to Kodak X-Omat AR film for 1 to 3 days, and the radioactivity on the filters was quantified (BAS 2000 II, Fuji Film, Tokyo, Japan). The cDNA clones of rat apo A-I (0.9 kb *Pst*I fragment),[24] mouse apo E (1.1 kb *Eco*RI fragment),[25] rat CYP2B1 (1.6 kb *Pst*I fragment),[26] and rat CYP1A2 (0.52 kb *Pst*I fragment)[27] were labeled with [5′α-^{32}P]dCTP using Megaprime DNA labeling system (Amersham, USA).

Biochemical analysis

Cholesterol levels were determined with a commercially available kit (Boehringer Mannheim, Germany). The serum level of apo A-I was quantified by densitometric scanning of nonreducing SDS-PAGE patterns of serum proteins.[28]

Statistical analysis

The statistical significance of differences among values was analyzed by one-way analysis of variance. When the result of treatment was significant, Duncan's multiple-range test or Student's *t*-test was performed.

Results

Dose-dependent induction of apo A-I gene expression in the liver by PCB (Experiment 1)

Body weight gain and relative liver weight are shown in *Table 1*. Body weight gain was not affected by dietary PCB, and liver weight was increased by PCB in a dose-dependent manner (*Table 1*). Serum levels of cholesterol and apo A-I were elevated by PCB in a dose-dependent manner (*Figure 1A and 1B*). Hepatic mRNA level of apo A-I was increased

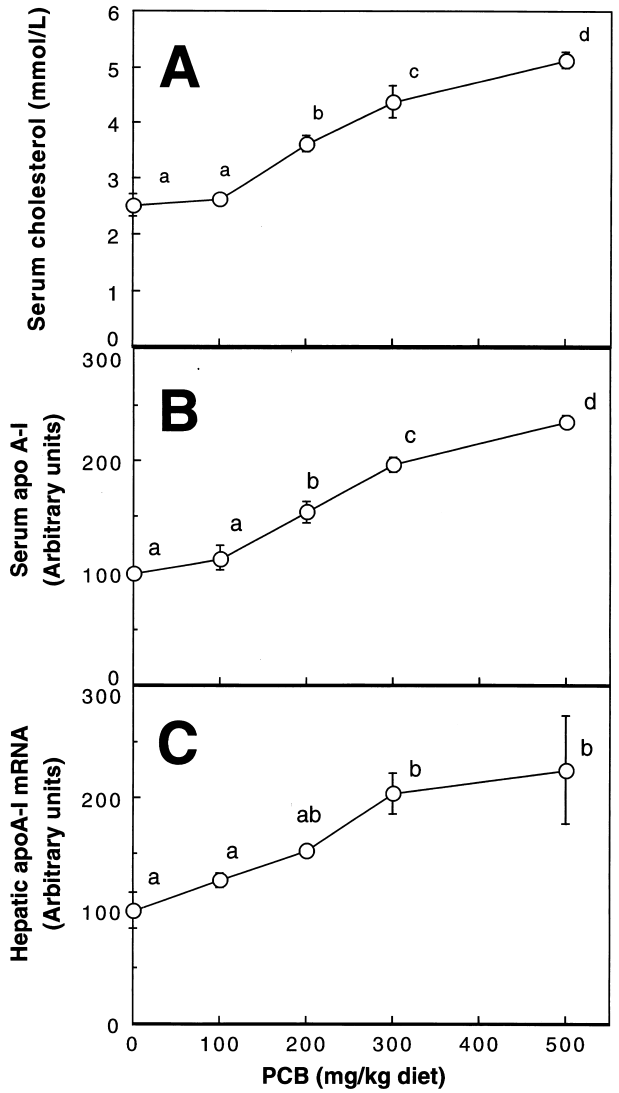

Figure 1 Dose-response of polychlorinated biphenyl (PCB) effect on serum level of cholesterol (A), serum level of apolipoprotein A-I (apo A-I; B), and hepatic apo A-I mRNA level (C) in rats (Experiment 1). Rats were fed the indicated amount of PCB for 14 days. B: Serum level of apo A-I was determined by nonreducing SDS-PAGE. Values of the control rats without PCB were set to 100. C: Fifteen micrograms of total RNA from liver were used for Northern blot analysis. RNA was equally loaded by observation of 28S and 18S ribosomal RNA stained with ethidium bromide. Quantitative analysis of specific hybridization was performed with BAS 2000 II. Because apo E mRNA level was not changed by PCB, values of apo A-I mRNA were normalized with those of apo E mRNA. Values of the control rats without PCB were set to 100. Values are the mean ± SEM for 4 rats. The statistical significance of differences among values was analyzed by one-way analysis of variance. When the result of the treatment was significant, Duncan's multiple-range test was performed. Points not followed by the same letter are significantly different ($P < 0.05$).

by PCB in a fashion similar to serum apo A-I (*Figure 1C*). When rats were fed a diet containing 500 mg/kg diet of PCB for 14 days, the levels of serum cholesterol, serum apo A-I, and hepatic apo A-I mRNA was 2.04-, 2.35-, and 2.25-fold higher, respectively, than those in control rats without treatment of PCB.

Table 2 Effect of dietary polychlorinated biphenyls (PCB; 200 mg/kg diet) and feeding period on body weight gain and liver weight in rats (Experiment 2)

Feeding period (days)	Control diet	PCB
	Body weight gain (g)	
1	4 ± 0	5 ± 1
3	15 ± 1	14 ± 1
7	31 ± 1	33 ± 1
14	62 ± 3	63 ± 1
60	202 ± 8	198 ± 4
105	212 ± 8	212 ± 11
	Liver weight (g/100 g body weight)	
1	4.65 ± 0.06	4.97 ± 0.25
3	4.70 ± 0.16	5.72 ± 0.08*,†
7	4.59 ± 0.07	6.45 ± 0.10†
14	4.74 ± 0.06	7.17 ± 0.08†
60	3.87 ± 0.05	6.86 ± 0.13†
105	3.56 ± 0.07	6.26 ± 0.15†

Values are the mean ± SEM for 6 rats.
*The statistical significance of differences between values was analyzed by one-way analysis of variance and Student's *t*-test, †$P < 0.001$.

Time-dependent induction of apo A-I gene expression in the liver by PCB (Experiment 2)

To investigate the time-course effect of PCB on levels of serum cholesterol, serum apo A-I, and hepatic apo A-I mRNA, rats were fed a diet containing 200 mg/kg diet of PCB for 1, 3, 7, 14, 60, and 105 days. Dietary PCB did not have any effect on body weight gain throughout the experimental period, and liver weight was gradually increased by 1.76-fold on Day 105 (*Table 2*). Serum level of cholesterol increased with age (*Figure 2*). PCB increased the serum cholesterol level by 1.69-fold within 14 days, and by 2.08-fold on Day 105 (*Figure 2A*). On the other hand, serum apo A-I level increased by 1.81-fold on Day 14 and by 3.31-fold on Day 105 (*Figure 2B*). Although hepatic apo A-I mRNA level was not increased significantly by PCB on Day 14 (1.30-fold), the level was increased by 3.99-fold on Day 105. Dietary PCB also induced CYP1A1/1A2 and CYP2B1/2B2 gene expressions (data not shown).[29,30]

Acute effect of xenobiotics on hepatic apo A-I gene expression (Experiment 3)

Because it was reported that PB, gemfibrozil, and gramoxone induced apo A-I gene expression acutely,[31–34] we examined acute effect of PCB, MC, and PB on apo A-I gene expression in rats. Body weight gain was lower in rats treated with PCB and MC than that in controls and PB-treated rats, and liver weight was higher in rats treated with PCB than in other groups (*Table 3*). These treatments had no effect on serum level of cholesterol (*Table 3*). Although serum level of apo A-I was a little higher in rats treated with PCB and PB than in controls ($P < 0.05$), hepatic mRNA level of apo A-I was not increased by treatment of PCB, PB, and MC (*Figure 3A*). On the other hand, the treatment of xenobiotics strongly induced CYP gene expressions (*Figure 3B*). MC and PB strongly induced CYP1A1/1A2 and CYP2B1/2B2 gene expression, respectively, and PCB induced both types of CYP gene expression.

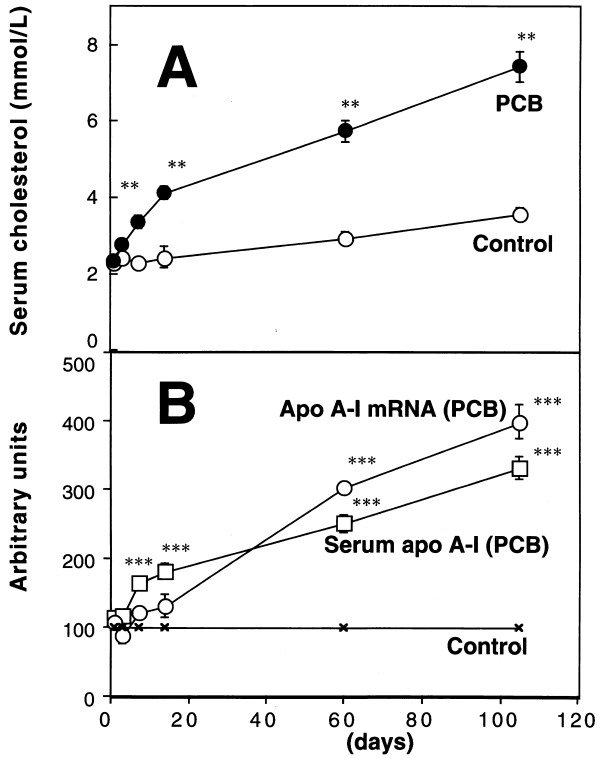

Figure 2 Time-course of polychlorinated biphenyl (PCB) effect on serum level of cholesterol (A), serum apolipoprotein A-I (apo A-I; B), and hepatic apo A-I mRNA (B) in rats (Experiment 2). Rats were fed 200 mg/kg diet of PCB for the indicated periods. B: Serum level of apo A-I was determined by nonreducing SDS-PAGE. Fifteen micrograms of total RNA from liver were used for Northern blot analysis. RNA was equally loaded by observation of 28S and 18S ribosomal RNA stained with ethidium bromide. Quantitative analysis of specific hybridization was performed with BAS 2000 II. Because apo E mRNA level was not changed by PCB, values of apo A-I mRNA were normalized with those of apo E mRNA. Values of the control rats without PCB in each period were set to 100. Values are the mean ± SEM for 6 rats. The statistical significance of differences between control group and PCB group was analyzed by one-way analysis of variance and Student's *t*-test, $^{***}P < 0.001$.

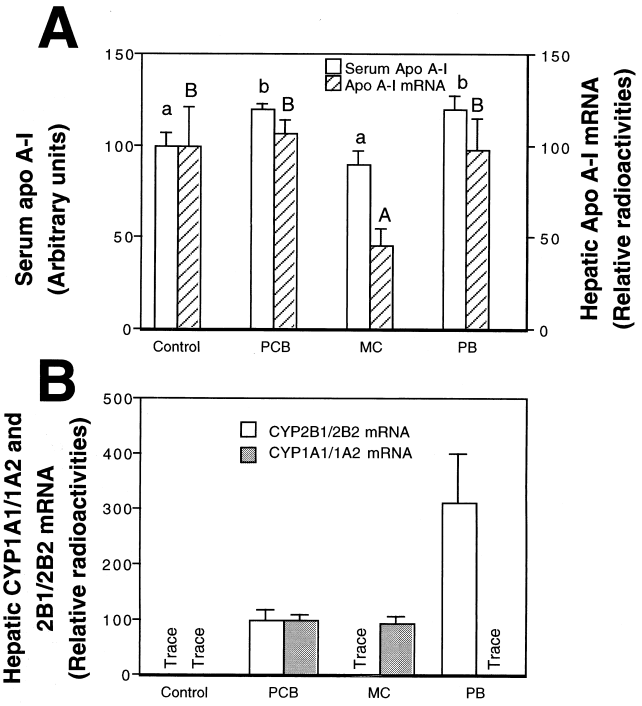

Figure 3 Acute effect of xenobiotics on serum level of apolipoprotein A-I (apo A-I; A), hepatic apo A-I mRNA level (A), and hepatic mRNA levels of cytochrome P-450 (CYP)1A1/1A2 and CYP2B1/2B2 (B) in rats (Experiment 3). Rats were injected intraperitoneally with 100 mg/kg body weight of phenobarbital (PB), 100 mg/kg body weight of PCB, and 25 mg/kg body weight of 3-methylcholanthrene (MC) at 6:00 PM. Livers of the rats were removed 16 hr after the injection. A: Serum level of apo A-I was determined by nonreducing SDS-PAGE. Fifteen micrograms of total RNA from liver were used for Northern blot analysis. RNA was equally loaded by observation of 28S and 18S ribosomal RNA stained with ethidium bromide. Quantitative analysis of specific hybridization was performed with BAS 2000 II. Values of the control rats without xenobiotics were set to 100. Values are the mean ± SEM for 4 rats. The statistical significance of differences among values was analyzed by one-way analysis of variance. When the result of the treatment was significant, Duncan's multiple-range test was performed. Bars not followed by the same letter are significantly different ($P < 0.05$). B: Fifteen micrograms of total RNA from liver were used for Northern blot analysis. RNA was equally loaded by observation of 28S and 18S ribosomal RNA stained with ethidium bromide. Quantitative analysis of specific hybridization was performed with BAS 2000 II. Values of the rats treated with PCB were set to 100. Values are the mean ± SEM for 4 rats.

Discussion

PCB-induced hypercholesterolemia was characterized by high levels of HDL-C and apo A-I[4,5] and cholesterol-rich VLDL.[6] The increased cholesterol biosynthesis has been thought to be one reason for PCB-induced hypercholesterolemia.[21,22] Here, we investigated hepatic apo A-I gene expression in rats treated with PCB and the relationship between apo A-I gene expression and drug-metabolizing enzymes. The treatment of PCB induced apo A-I gene expression in the liver in dose- and time-dependent manners (*Figures 1 and 2*). Serum apo A-I level was closely dependent upon apo A-I mRNA level in the liver. These results indicate that the induction of apo A-I gene expression by PCB would be responsible for the high level apo A-I in serum and hyper-alpha-cholesterolemia in rats fed PCB. Therefore, the coordinative inductions of both apo A-I and HMG-CoA reductase gene expression by PCB result in hypercholesterolemia.[20,21]

To elucidate the mechanism by which apo A-I gene expression is induced, the acute effect of xenobiotics on apo A-I gene expression has been investigated.[31–34] Chao et al.[31] reported that PB treatment increased apo A-I mRNA level by 10-fold within 16 hr in rat liver. Tam and Deeley[32] also observed that PB stimulated apo A-I gene transcription in Hep3B cells. Treatment of gemfibrozil, a hypolipidemic drug, to HepG2 cells resulted in a 2-fold induction of apo A-I gene expression.[33] It was reported that there were two drug-response elements in the promoter region of human apo A-I gene.[33] These reports suggested that some drugs acutely induced apo A-I gene expression through a direct pathway to the gene. Contrary to these reports, the acute treatment of PCB, PB, and MC in our study did not induce apo A-I gene expression in the liver, although these xenobiotics induced CYP gene expressions (*Figure 3*). The

Table 3 Acute effect of polychlorinated biphenyls (PCB), 3-methylcholanthrene (MC), and phenobarbital (PB) on body weight gain, liver weight, and serum cholesterol in rats (Experiment 3)

	Control	PCB	MC	PB
Body weight gain (g/day)	$6 \pm 0^{*,c}$	3 ± 1^{b}	-9 ± 1^{a}	6 ± 1^{c}
Liver weight (g/100 g body weight)	4.38 ± 0.06^{b}	4.65 ± 0.09^{c}	4.17 ± 0.05^{a}	$4.34 \pm 0.04^{a,b}$
Serum cholesterol (mmol/L)	2.04 ± 0.08	2.07 ± 0.05	1.94 ± 0.23	1.97 ± 0.10

Values are the mean ± SEM for 4 rats. Rats were treated intraperitoneally with 100 mg/kg body weight of PCB, 25 mg/kg body weight of MC, or 100 mg/kg body weight of PB at 6:00 PM for 16 hr.
*The statistical significance of differences among values was analyzed by one-way analysis of variance.
When the result of the treatment was significant, Duncan's multiple-range test was performed.
Means within a line not followed by the same letter are significantly different ($P < 0.05$).

conflicting results between those from Chao et al.[31] and ours are puzzling at present. We analyzed the data statistically; however, Chao et al.[31] presented only one sample in each group. We found more than 2-fold differences in apo A-I mRNA levels, even among untreated rats (unpublished data). We also tested the effect of PCB on apo A-I gene expression in rat primary hepatocytes. Although PCB-treatment somewhat increased apo A-I mRNA level in rat hepatocytes, it was lower than that observed in the liver.[29] As shown in *Figures 1 and 2*, hepatic apo A-I gene expression increased gradually during the treatment of PCB and depended upon the amount of PCB. Taken together, we supposed that apo A-I gene expression was induced indirectly by the accumulated PCB rather than by the direct stimulation of gene transcription.

The mechanism of apo A-I gene expression has been extensively investigated.[35–43] Retinoic acid, thyroid hormone, estrogen, glucocorticoid hormone, and insulin are known to change the transcription of apo A-I gene.[36–38,41–43] However, because the treatment of PCB to rats did not alter the serum level of insulin, glucagon, corticosterone, and triiodothyronine (Nagaoka and Yoshida, unpublished results), it was postulated that PCB induced apo A-I gene expression by factors other than these hormones. Several transcription factors have been identified to control apo A-I gene transcription such as hepatocyte nuclear factor (HNF)-3 and HNF-4.[35,39,40] Among these liver-enriched transcription factors, HNF-4 is thought to be one of the most critical factors for hepatocyte differentiation functions.[44,45] HNF-4 belongs to the nuclear receptor superfamily. Specific ligand for HNF-4 has not been identified. Recently, Hertz et al.[46] demonstrated that fatty acyl-CoAs were possible ligands of HNF-4. The transactivation of HNF-4 was dependent upon the length and saturation of fatty acyl-CoAs. On the other hand, dietary fat is also known to change apo A-I gene expression.[47] The treatment of PCB to rats showed hepatic hypertrophy and proliferated endoplasmic membranes; PCB stimulated not only cholesterol biosynthesis but also fatty acid synthesis.[48] Lipid metabolism and fatty acid composition in hepatocytes might be altered by PCB. Therefore, it may be possible to hypothesize that modulation of HNF-4 activity by alteration of hepatic lipid composition in rats fed PCB might be responsible for the induction of apo A-I gene expression by PCB. Miyazaki et al.[49] reported that long-term treatment of PB to rat hepatocytes induced the DNA binding activity of HNF-4. Although we did not find any induction of HNF-4 gene expression by PB-treatment for 24 hr (unpublished results), long-term treatment of xenobiotics such as PCB and PB might induce HNF-4 gene expression and, subsequently, induce apo A-I gene expression.

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