Persistent Exposure to 2,3′,4,4′,5-Pentachlorobiphenyl (PCB118) Induces Hyperalphacholesterolemia in Rats

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Polychlorinated biphenyls (PCBs) are synthetic organic compounds with two phenyl groups well known environmental pollutants. This study examined the effect of persistent exposure to 2,3′,4,4′,5-pentachlorobiphenyl (PCB118) on serum cholesterol levels in male rats. Male Sprague Dawley rats were administered weekly intraperitoneal injections of either PCB118 (20 mg/kg) dissolved in corn oil or corn oil alone. One week after 2 and 5 administrations, the rats were sacrificed by a pentobarbital injection, and the effect of PCB118 on the serum levels of total cholesterol, high density lipoprotein-cholesterol (HDL-cholesterol), and low density lipoprotein-cholesterol (LDL-cholesterol) was investigated. The protein expression level of apolipoprotein A-I (apo A-I) and 3-hydroxy-3-methylglulutaryl coenzyme A (HMG-CoA) reductase was also examined. In this study, the administration of PCB118 induced hyperalphacholesterolemia in rats. In addition, the protein expression level of HMG-CoA reductase and apo A-I was higher in the PCB118-treated rats than in the control. These results suggest that the hyperalphacholesterolemia induced by PCB118 in male rats may be associated with an increase in HMG-CoA reductase and apo A-I expression.

Key words —— 2,3′,4,4′,5-pentachlorobiphenyl, hyperalphacholesterolemia, apolipoprotein A-I

INTRODUCTION

Polychlorinated biphenyls (PCBs) are synthetic organic compounds with two phenyl groups and widespread environmental pollutants. They comprise a family of 209 congeners based on the position of the attached chlorine atoms. PCBs were produced industrially on account of their high stability and low water solubility. However, their production was banned in the 1970s due to concerns about their high toxicity and persistence in the environment. They can accumulate in various tissues in humans and animals through the food chain and cause a variety of health problems.

Previous studies on PCBs reported that they produced toxic responses in many biological systems. Exposure to PCBs resulted in an increased mortality rate from stomach and intestinal cancer.[1] PCBs are toxic to the nervous system.[2,3] In addition, PCBs disturb the endocrine functions including gonadal and thyroid functions.[4–7]

Studies on PCBs reported that they had adverse effects on the serum cholesterol levels. Exposure of female rats to PCB126 (non-ortho-PCB) resulted in increased levels of serum total cholesterol and high density lipoprotein-cholesterol (HDL-cholesterol).[8] Exposure of male and female rats to PCB105 (ortho-PCB) increased the serum total cholesterol level but did not examine the effect on HDL-cholesterol.[9] Oda *et al.*[10,11] also showed increased levels of apolipoprotein A-I (apo A-I) and 3-hydroxy-3-methylglulutaryl coenzyme A (HMG-CoA) reductase in PCB-treated rats, and suggested that the hyperalphacholesterolemia may be caused by the induced levels of apo A-I and HMG-CoA reductase.

HDLs are the smallest and densest of the

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lipoproteins, and deliver cholesterol from the tissues to the liver for recycling or excretion in bile. They consist of a hydrophobic core surrounded by phopholipids, unesterified cholesterol and apolipoproteins.[12] Apo A-I is synthesized in the liver and intestine, and is the major apolipoprotein component of HDL in the bloodstream.[13, 14] Cholesterol is synthesized mainly from acetyl CoA and acetoacetyl CoA through HMG-CoA reductase, which is the regulatory enzyme of this pathway.[15] Cholesterol synthesis occurs in most cells but its production is the highest in the liver.

In previous studies, 2,3′,4,4′,5-pentachlorobiphenyl (PCB118) is among the congeners frequently found in wildlife and human.[16, 17] And the effect of PCB118 (ortho-PCB) on serum cholesterol levels has not investigated. Therefore, this study examined the effect of persistent exposure to PCB118 on the serum level of total cholesterol, HDL-cholesterol, and low density lipoprotein-cholesterol (LDL-cholesterol). In addition, the protein expression levels of serum apo A-I, hepatic apo A-I and hepatic HMG-CoA reductase were examined to determine the mechanism of PCB118-mediated hyperalphacholesterolemia.

MATERIALS AND METHODS

Animals and Treatments —— Six week old Sprague Dawley male rats were used in this study. The rats were housed in cages with a 12 hr light/dark cycle at 21 ± 2°C and 50 ± 5% humidity, and were given a commercial diet (#55VXT0038, Samyang, Seoul, Korea) and water *ad libitum*. PCB118 were obtained from Dr. Ehrenstorfer Company (Augsburg, Germany). The purity of the PCB118 was 99.0%. Stock solutions were prepared by dissolving PCB118 in *n*-hexane. The PCB stock solution was added to corn oil and vortexed. The *n*-hexane was then removed by evaporation. The rats were divided into 2 groups: the PCB118-treated, and control. Each group was subdivided into 2 groups: 2 and 5 injections groups. A total of 20 rats were divided into 4 groups containing 5 rats each. The rats in each group received weekly intraperitoneal injections of either PCB118 (20 mg/kg) dissolved in corn oil or the corn oil alone (control). One week after 2 and 5 administrations, the animals were sacrificed by a pentobarbital injection. Serum and liver were collected and the samples were stored at −70°C until needed. The animal experiment was performed according to Gyeongsang National University Experimental Animals guideline.

Biochemical Analysis —— Total cholesterol, triglyceride, glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) were measured by using a Total cholesterol kit, Triglyceride kit, GOT/GPT kit (Bio Clinical System, Anyang, Korea). HDL-cholesterol level was enzymatically determined by using a commercial kit (HDL-cholesterol, ASAN Pharmaceutical, Hwaseong, Korea). LDL-cholesterol level was obtained by the method of Friedewald *et al.*[18] The formula is as follows:

LDL-cholesterol
= Total cholesterol − (HDL-cholesterol
+ Triglyceride/5)

Two Dimensional Electrophoresis —— The serum was treated with a Montage albumin depletion kit (Millipore, Billerica, MA, U.S.A.) according to the manufacturer's instructions. 50 µl of the processed sample was collected, and treated with 0.9 ml cold acetone (−20°C) for 2 hr. All samples were centrifuged at 13000 rpm and 4°C for 10 min. The supernatant was removed and the protein pellets were dried in a lyophilizer. The dried pellets were dissolved in a sample buffer containing 7 M urea (BIO BASIC INC., Ontario, Canada), 2 M thiourea (MERCK, Darmstadt, Germany), 4% (w/v) 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS, Sigma, St. Louis, MO, U.S.A.), 60 mM dithiothreitol (DTT, Promega, Madison, WI, U.S.A.), and a 0.5% (v/v) immobilized pH gradient (IPG) buffer (GE Healthcare Life Sciences, Uppsala, Sweden). The lysate was then maintained in an ice slurry for 30 min. The samples were then centrifuged at 13000 rpm for 30 min at 4°C. The supernatant was transferred to an eppendorf tube and stored at −70°C until needed. The protein concentration was estimated using a Bradford protein assay kit (Biorad, Hercules, CA, U.S.A.).

Isoelectric focusing (IEF) was performed using an Ettan IPGphor 3 (GE Healthcare Life Sciences). The samples were mixed with the appropriate amount of a rehydration buffer containing 7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 60 mM DTT, 0.5% (v/v) IPG buffer and 0.002% (w/v) bromophenol blue (Sigma), and applied to an IPG strip (Immobiline DryStrip™, 13 cm, GE Healthcare Life Sciences). The protein samples were focused for a total of 83.8 kVh. After IEF, the IPG strips were equilibrated with 10 mg/ml DTT in an equilibration

buffer [6 M urea, 2% (w/v) sodium dodecyl sulphate (SDS, Sigma), 30% (v/v) glycerol (Sigma), 0.002% (w/v) bromophenol blue, and 50 mM Tris-HCl, pH 8.8] for 15 min, and then incubated in the same buffer for a further 15 min, replacing the DTT with 40 mg/ml iodoacetamide (Sigma). After equilibration, the IPG strips were placed onto 12% SDS-polyacrylamide gel (160 × 160 × 1 mm), and sealed with 0.5% (w/v) agarose (Promega). The gels were run at 10 mA/gel for 15 min for initial migration. The current was then increased to 20 mA/gel for separation until the dye reached the bottom of the gel.

Silver staining was performed according to the method reported by Mortz *et al.*[19] The stained spots were digitalized using an Agfa Arcus 1200^{TM} image scanner (Agfa-Gevaert, Mortsel, Belgium), and the acquired images were analyzed using Phoretix^{TM} 2D software (Ver. 5.01, NonLinear Dynamics, Newcastle, U.K.).

Matrix-assisted Laser Desorption/Ionization-time of Flight Mass Spectrometry (MALDI-TOF MS) and Database Search —— In-gel digestion of the protein spots on the gels stained with silver nitrate was performed essentially as described by Shevchenko *et al.*[20] After staining, the spots of interest were chosen using a 1 mm diameter micropipette tip. The gel pieces were washed with distilled water, followed by 50% acetonitrile (MERCK), and dried completely in a vacuum centrifuge. The dried gel pieces were then rehydrated in 10 mM DTT/100 mM NH_4HCO_3 (Sigma), and incubated at room temperature for 45 min. The rehydrated gel pieces were transferred to a 55 mM iodoacetamide solution in 100 mM NH_4HCO_3, and incubated in the dark for 30 min at room temperature. The gel pieces were dried, rehydrated in a digestion buffer containing 50 mM NH_4HCO_3, 5 mM $CaCl_2$ (Sigma), 12.5 μg/ml porcine trypsin (Promega), and incubated for 45 min on ice. The excess liquid was removed, and a 10 μl digestion buffer was added without trypsin. After overnight digestion with trypsin (approximately 16 hr) at 37°C, the supernatants were recovered and extracted twice in a 1 : 1 (v/v) mixture containing 5% formic acid and acetonitrile. The extracts were then pooled and dried in a vacuum centrifuge.

Dried tryptic peptides were redissolved in a 2 μl solution containing distilled water (DW), acetonitrile, and trifluoroacetic acid (MERCK, 93 : 5 : 2, v/v/v). A solution-phase nitrocellulose (NC, Sigma) method was used according to Landry *et al.*[21] α-Cyano-4-hydroxycinnamic acid (40 mg/ml, Sigma) and NC (20 mg/ml, Millipore) was prepared separately in acetone (MERCK) and mixed with isopropanol (MERCK) at a 2 : 1 : 1 ratio, respectively. The internal standards of neurotensin (monoisotopic mass, 1672.9175, Sigma) and angiotensin I (1296.6853, Sigma) were added to the mixture to make a matrix solution. The resulting solution was then mixed at a 1 : 1 ratio with the sample peptide prepared by trypsin digestion. One microliter of the mixed solution was spotted onto a target circle plate and dried. The dried samples were washed sequentially with 5% formic acid (MERCK) and DW, and then allowed to dry completely. The dried spot sample on the target was analyzed by Voyager-DE STR MALDI-TOF mass spectrometry (PerSeptive Biosystems, Framingham, MA, U.S.A.). The masses of the parent ions were measured in a positive ion reflections/delayed extraction mode with an accelerating voltage of 20 kV, a grid voltage of 65% and a delay time of 100 ns. The mass spectra were acquired as the ion signals were generated by irradiating the target with 128 laser pulses. Monoisotopic peptide masses were selected in the mass range of 800–2500 Da. The proteins were identified by peptide mass fingerprinting (PMF) using the Matrix Science — Mascot program (http://matrixscience.com) and the National Center for Biotechnology Information (NCBI) protein sequence database (http://ncbi.nlm.Nih.gov). The peptide mass tolerance was 50 ppm and one missed cleavage site was allowed. The carbamidomethylation of cysteines and oxidation of methionine were considered as possible modifications during the search. The extent of sequence coverage, the number of peptides matched, and the protein score with significant probability were considered before accepting the identification.

Western Blotting —— 5 μl of serum was mixed with 75 μl of DW and then 20 μl of 5 × sample buffer [60 mM Tris-HCl (pH 6.8), 25% glycerol, 2% SDS, 14.4 mM 2-mercaptoethanol (Sigma), a few grains of bromophenolblue] was added. The mixed sample was boiled for 5 min. 4 μl of the sample was loaded onto the well and separated in 12% polyacrylamide gel.

25 mg of liver tissue was added to the lysis buffer, which consisted of 0.05 M Tris-HCl (pH 8.0), 1.5 M sodium chloride (Sigma), 0.02% sodium azide (Sigma), 0.1% SDS, 1% NP-40 (Sigma), 0.05% sodium deoxycholate (Sigma) and a protease inhibiter (Halt^{TM} Protease Inhibitor Cock-

tail Kit, Pierce, Rockford, IL, U.S.A.). The samples were then sonicated for 6 min (total processing time: pursing for 3 min, resting for 3 min) in an ice slurry. The homogenized sample was centrifuged at 13000 rpm at 4°C for 30 min. The clear supernatant was then transferred to an Eppendorf tube and stored at −70°C until needed. The protein concentration was estimated using a Bradford protein assay kit (Biorad). The sample was mixed with 5 × sample buffer [60 mM Tris-HCl (pH 6.8), 25% glycerol, 2% SDS, 14.4 mM 2-mercaptoethanol, a few grains of bromophenol blue], and the mixed sample was boiled for 5 min. The sample underwent electrophoreses through a 12% polyacrylamide gel.

After running, the gel was transferred to a polyvinyldene fluoride (PVDF) membrane (Immobilon-P, 0.45 mm, Millipore) using the TE 77 Semi-Dry Transfer Unit (GE Healthcare Life Sciences). The PVDF membrane was blocked with 5% skim milk in phosphate buffered saline (pH 7.4) for 30 min. The membrane was then incubated for 90 min with the primary antibody (apo A-I and HMG-CoA reductase (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.), Albumin (MP Biomedicals, Solon, OH, U.S.A.), β-actin (Millipore, Billerica, MA, U.S.A.)). After washing with 0.05% phosphate buffered saline-Tween20 (PBST, pH 7.4), the membrane was incubated for 1 hr with the secondary antibody (anti-rabbit IgG-HRP (Dako, Glostrup, Denmark), anti-goat IgG-HRP and anti-mouse IgG-HRP (Santa Cruz Biotechnology)). After washing again with 0.05% PBST, the membrane was soaked in an ECL Western Blotting Detection Reagents (GE Healthcare Life Sciences) and exposed to X-ray film (FUJIFILM, Tokyo, Japan).

Statistical Analysis —— A *t*-test was used to analyze the data. All statistical analyses were carried out using the SPSS 14.0 program.

RESULTS

Biochemical Analysis

The effect of PCB on body weight, serum GOT/GPT, total cholesterol, HDL-cholesterol and LDL-cholesterol level was examined by administering rats weekly doses of PCB118 (20 mg/kg) and corn oil (control). One week after 2 and 5 administrations, the animals were sacrificed. Table 1 shows the changes in body weight and serum GOT/GPT levels after the administration of PCB118 in rats. There was a significantly lower body weight in the PCB118-treated rats given 5 injections than the control ($p < 0.05$). However, there was no significant differences of body weight in the rats given 2 injections. The serum GOT/GPT levels were similar in all groups. Table 2 shows the changes in the total cholesterol, HDL-cholesterol and LDL-cholesterol levels. In the 5 injections group, the total cholesterol and HDL-cholesterol levels of the PCB118-treated rats were significantly higher than those of the control but there was no change in the 2 injections group (Table 2). In contrast to HDL-cholesterol, the LDL-cholesterol levels were relatively unaffected in all groups (Table 2).

Table 1. Effects of Exposure to PCB118 on the Body Weight and GOT/GPT Levels

Parameter	Group	Injections (Weekly) 2	5
Body weight (mg)	Control	145 ± 2.5	319 ± 27.6
	PCB118	153 ± 17.4	212 ± 9.5*
GOT (IU/l)	Control	83 ± 9.1	147 ± 28.3
	PCB118	91 ± 19.6	172 ± 8.5
GPT (IU/l)	Control	27 ± 5.7	34 ± 9.0
	PCB118	25 ± 5.0	39 ± 11.8

Note. The values are represented as the mean ± S.D. *Significantly different from the control values at $p < 0.05$.

Table 2. Effects of Exposure to PCB118 on the Total Cholesterol, HDL-cholesterol and LDL-cholesterol Levels

Parameter	Group	Injections (Weekly) 2	5
Total cholesterol (mg/dl)	Control	109.33 ± 17.45	83.90 ± 6.76
	PCB118	129.02 ± 10.28	99.22 ± 4.94*
HDL-cholesterol (mg/dl)	Control	58.30 ± 5.92	41.53 ± 4.56
	PCB118	61.33 ± 5.51	52.43 ± 6.75*
LDL-cholesterol (mg/dl)	Control	43.25 ± 11.59	35.33 ± 8.57
	PCB118	61.27 ± 12.58	38.97 ± 3.97

Note. The values are represented as the mean ± S.D. *Significantly different from the control values at $p < 0.05$.

Two dimensional electrophoresis (2-DE) and MALDI-TOF MS

2-DE was used to compare the differential expression of the proteins in the control and PCB118-treated rat sera. Figure 1 shows the 2-DE gel images of the PCB118-treated (20 mg/kg) and control rat sera in the 5 injections groups. The rat serum 2-DE profiles were obtained using the data from the silver-stained gels of 100 μg total proteins. Figure 1 shows the 2-DE pattern of the rat serum proteome obtained using pH 4–7 IPG strips and 12% polyacrylamide gel. Triplicate samples for 2-DE were prepared. The 2-DE patterns were highly reproducible when the protein patterns on the same sample were compared. By comparing the two serological 2-DE reference maps, one spot showed differential expression between the control and PCB118-treated rats. The spots were excised from the gels, digested by trypsin, and the mass spectrum of the protein was then acquired by MALDI-TOF MS (Fig. 2). For PMF, the spectrum was applied to the matrix science-mascot search program and the protein spots were identified successfully as apo A-I (Accession No. 2145145 by NCBI) with the matched peptide, 7 and amino acid sequence coverage, 34% (data not shown).

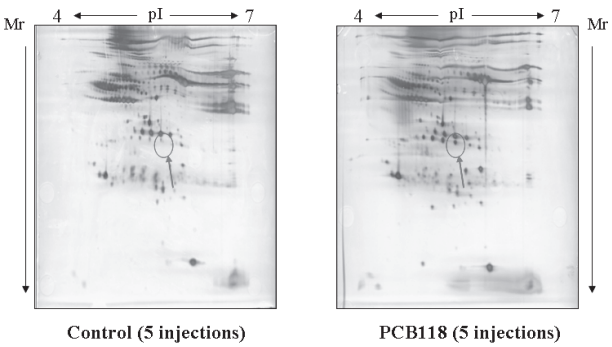

Fig. 1. Effect of Exposure to PCB118 on Expression of Serum Proteome in Rats Given 5 Injections

IEF was carried out at 83.8 kVh using a pH 4–7 IPG strip (13 cm) with a protein loading of 100 μg. SDS-polyacrylamide gel electrophoresis (PAGE) was performed on a 12% polyacrylamide gel and then stained with silver nitrate. In the PCB118-treated group (20 mg/kg), an up-regulated protein spot was detected (the arrow).

Western Blotting in the Liver Lysates

In order to confirm the effects of PCB118 detected by 2-DE analysis, the serum proteins were separated by SDS-polyacrylamide gel electrophoresis (PAGE), and the proteins were transferred onto a PVDF membrane. The density of the western blotting bands is represented as the relative value of serum apo A-I compared to serum albumin. The level of serum apo A-I expression was significantly higher in the PCB118-treated rats given 5 injections than in the control (Fig. 3).

The effect of PCB118 on the protein expression levels of hepatic apo A-I and HMG-CoA reductase was examined by separating the liver lysates by SDS-PAGE, and transferring the proteins onto a PVDF membrane. The density of the western blotting bands is represented as the relative value of hepatic apo A-I and HMG-CoA reductase compared to β-actin. The level of hepatic apo A-I expres-

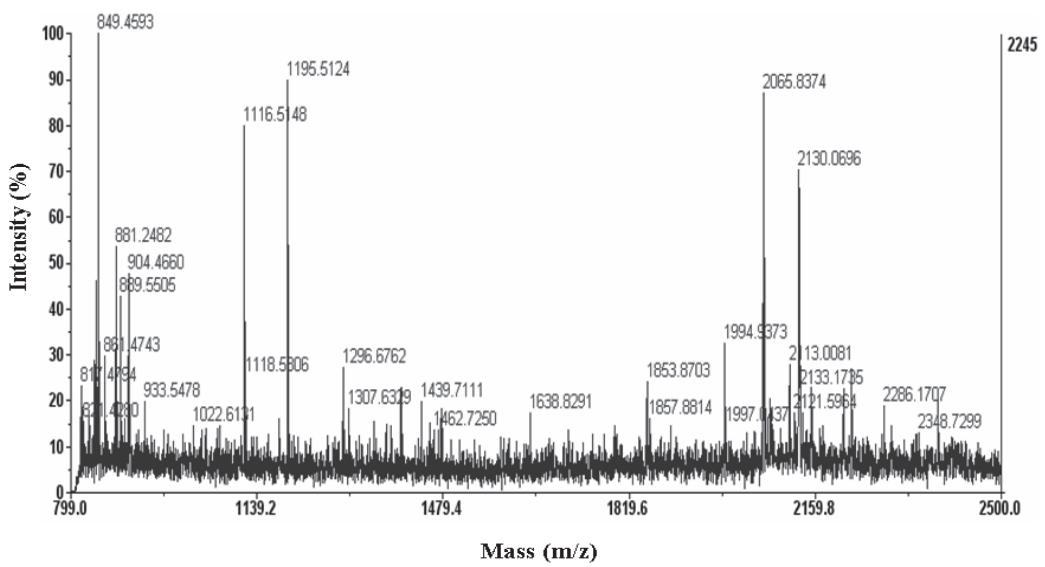

Fig. 2. Mass Spectrum of a Differentially Expressed Protein Spot (in Fig. 1) by MALDI-TOF MS

PMF was performed using the matrix science-mascot program and the protein spot was identified as apo A-I.

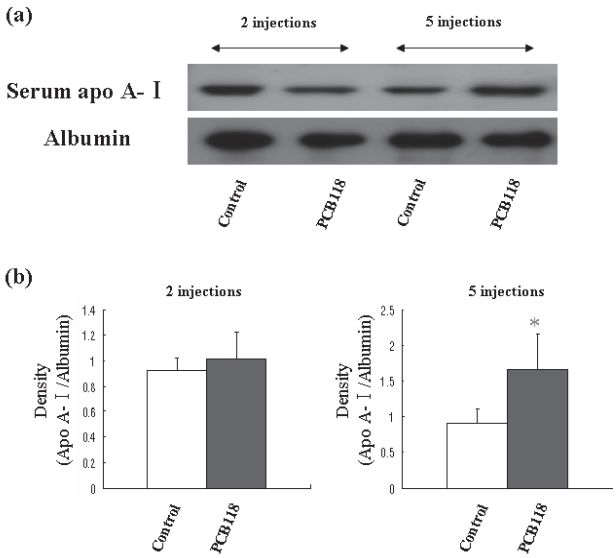

Fig. 3. Expression of Apo A-I in the Rat Serum after the Administration of Corn Oil (control) and PCB118 (20 mg/kg)

(a) Apo A-I and serum albumin were detected by western blotting. Serum albumin was used as the standard. (b) Density of western blotting bands is represented as mean ± S.D. of the ratio of apo A-I/albumin within each sample. The asterisks indicate significant differences from the controls ($p < 0.05$).

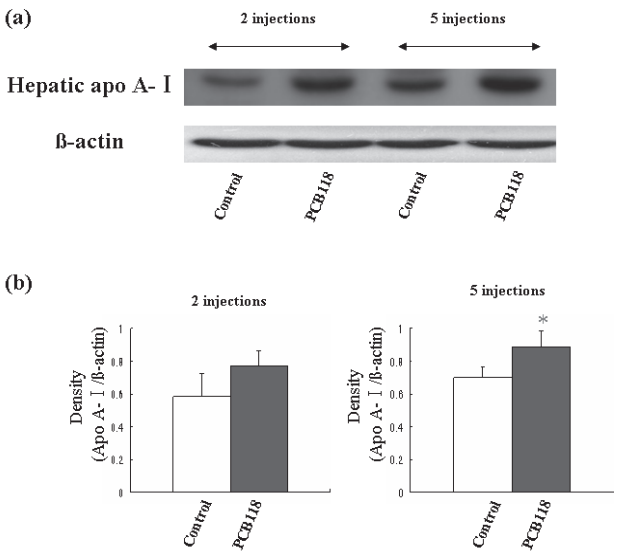

Fig. 4. Expression of Apo A-I in the Rat Liver after the Administration of Corn Oil (control) and PCB118 (20 mg/kg)

(a) Apo A-I and β-actin were detected by western blotting. β-actin was used as the standard. (b) Density of western blotting bands is represented as mean ± S.D. of the ratio of apo A-I/β-actin within each sample. The asterisks indicate significant differences from the controls ($p < 0.05$).

sion was significantly higher in the PCB118-treated rats given 5 injections than in the control. On the other hand, the level of expression in the 2 injections group was relatively unchanged (Fig. 4). The level of hepatic HMG-CoA reductase expression in the PCB118-treated rats given 5 injections was significantly higher than the control but there was no change in the 2 injections group (Fig. 5).

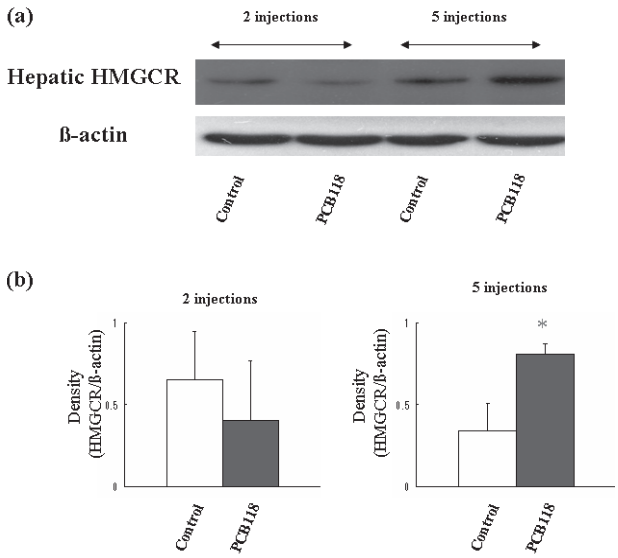

Fig. 5. Expression of HMG-CoA Reductase in the Rat Liver after the Administration of Corn Oil (control) and PCB118 (20 mg/kg)

(a) HMG-CoA reductase and β-actin were detected by western blotting. β-actin was used as the standard. (b) Density of western blotting bands is represented as mean ± S.D. of the ratio of HMG-CoA reductase/β-actin within each sample. The asterisks indicate significant differences from the controls ($p < 0.05$).

DISCUSSION

This study examined the effect of persistent exposure to a ortho-substituted PCB, PCB118 on the serum cholesterol levels in male rats. PCB118 administrations were repeated weekly to examine the effect of persistent exposure. In this study, the control values between 2 injections and 5 injections group are quite different on most parameters. Those differences might be due to repeated corn oil injection. Persistent exposure to PCB118 induced an increase in the total cholesterol and HDL-cholesterol level in rats given 5 injections. On the other hand, the LDL-cholesterol level was relatively unchanged. These results show that persistent exposure to PCB118 can cause of hyper-alpha-cholesterolemia. Hyperalphacholesterolemia is an increase in the HDL-cholesterol levels in the bloodstream not the LDL-cholesterol. HDLs are heterogeneous and can be distributed accord-

ing to their flotation density, immunological composition, molecular weight, or electrophoretic mobility. Most HDLs have 'α-mobility' in agarose gel electrophoresis.[12, 22] HDLs can remove cholesterol from the arteries and transport it to the liver. Many studies have shown that the increased HDL-cholesterol is protective against the development of cardiovascular disease.[23, 24] However, Van Acker *et al.*[25] reported that high HDL-cholesterol level does not protect against coronary artery disease when associated with the lowering gene variants of the cholesteryl ester transfer protein (CETP) and hepatic lipase (HL). In female rats treated with PCB126, the HDL-cholesterol and cardiovascular risk factors were increased all together.[8]

The levels of serum GOT/GPT were not significantly changed in all groups. These results represent that the rat liver has not damaged in this exposure levels of PCBs.

2-DE is a useful tool for the global analysis of gene expression at the protein level as well as for gaining a more complete understanding of various biological functions. This technique was originally described by O'Farrell[26] and Klose,[27] and separates the proteins according to their isoelectric point (pI) and molecular weight (Mr). 2-DE plays a key role in proteomics, and is used widely in comparative analysis of the protein expression levels. For example, the differential protein expression of murine macrophage RAW 264.7 cells induced by *Candida albicans* was investigated, and the differential expression of proteins between a normal liver and hepatocellular carcinoma tissue was examined as a potential diagnostic biomarker.[28, 29] In addition, Xu *et al.*[30] applied proteomic tools to examine the adverse effects on renal proteins in fluoride-treated rats. An analysis of the serum proteome is quite useful for detecting the pathological changes because an injury to the cells or tissues in the body will cause some leakage of the proteins into the bloodstream. Therefore, this study compared the serum 2-DE profiles of the control and PCB118-treated rats to obtain the information on the mechanism for PCB-mediated hyperalphacholesterolemia. The serum 2-DE gels were analyzed for screening of differentially expressed proteins in the sera from PCB118-treated and control rats. In the serum 2-DE profiles of the PCB118-treated rats given 5 injections, the expression of a protein spot was higher than the control. The mass spectrum of the protein spot was acquired by MALDI-TOF MS. The protein spot was identified successfully as apo A-I (Accession No. 2145145 by NCBI). In a large number of serum proteins, only one protein, apo A-I was screened out.

Similarly, with 2-DE, the results of Western blotting showed higher serum apo A-I expression in the PCB118-treated rats given 5 injections than the control. In addition, the expression of hepatic apo A-I and HMG-CoA reductase was also induced in the PCB118-treated rats given 5 injections. These results show that persistent exposure to PCB118 increases the synthesis and secretion of apo A-I as well as the cholesterol biosynthesis by hepatic HMG-CoA reductase.

In previous reports, a study on exposure to PCB105 in rats revealed hypercholesterolemia, but did not examine the HDL-cholesterol levels.[9] Oda *et al.*[11] suggested that the induction of hepatic apo A-I mRNA and cholesterol biosynthesis in rats treated with Aroclor 1248 might be responsible for the hyperalphacholesterolemia. However, their studies were not investigated sufficiently for the HDL-cholesterol level and HMG-CoA reductase, which is the critical factor for cholesterol synthesis in liver. These results revealed consistent increased levels of hepatic/serum apo A-I, HMG-CoA reductase, total cholesterol and HDL-cholesterol in rats given 5 injections. PCB118 is even less toxic than non-ortho-PCB according to the toxic equivalency factors.[31] However, persistent exposure to PCB118 induced apo A-I, HMG-CoA reductase and hyperalphacholesterolemia. In addition, Oda *et al.*[11] suggested that the gene expression of apo A-I is induced indirectly by the accumulated PCB rather than by direct stimulation of the gene by PCB. In this study, it is unclear that the gene expression of apo A-I is indirectly induced by the accumulation of PCB118. It is believed that the induction of HMG-CoA reductase and apo A-I is involved in PCB118-mediated hyperalphacholesterolemia.

PCBs are classified mainly into two congeners, ortho-PCBs and non-ortho-PCBs, according to the position of the substituted chlorine atoms. Studies on PCBs have been performed mainly using non-ortho-PCBs (PCB126 *etc.*) or PCB mixtures (Aroclor 1248 *etc.*). There have been relatively few studies on ortho-PCB, such as PCB118. This paper report for the first time that PCB118 induces hyperalphacholesterolemia. In previous studies, hyperalphacholesterolemia was caused by non-ortho-PCB and PCB mixtures.[8, 32] In addition, PCB118, ortho-PCB also induces hyperalphacholesterolemia. Although many congeners have not been investigated,

it is believed that the PCB-mediated hyperalphacholesterolemia is apart from structural features of PCB.

A mechanism for regulating the HDL-cholesterol level is complicated and not incompletely understood. Among the factors involved the process, CETP facilitates the transfer of the cholesteryl ester from HDL to other lipoproteins and lecithin: cholesterol acyltransferase (LCAT) produces spherical HDL particles. Adenosine triphosphate (ATP)-binding cassette A1 (ABCA1), ATP-binding cassette G1 (ABCG1) and scavenger receptor B-1 (SR-B1) promote the efflux of cellular cholesterol to the extracellular space where they are picked up by apo A-I.[12, 33] Therefore, further study of the associated factors will be needed to obtain a better understanding of the relationship between the HDL-cholesterol level and PCBs.

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