Role of the Dioxin-like Toxic Compound Coplanar Polychlorinated Biphenyl, 3,3′,4,4′,5-Pentachlorobiphenyl in Reducing Hepatic Alcohol Dehydrogenase Levels in Rats *in Vivo*

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The ability of a dioxin-like toxic compound, coplanar polychlorinated biphenyl, 3,3′,4,4′,5-pentachlorobiphenyl (PCB126) to reduce the protein level of hepatic class I alcohol dehydrogenase (ADH), which plays an important role in the metabolism of ethanol, was studied. Male Wistar rats received PCB126 25 mg/kg i.p. At this dose the compound induces a wasting syndrome. PCB126 administration resulted in a significant suppression of the protein level of class I ADH, whereas the difference between free- and pair-fed controls was slight. These results suggest that dioxins also reduce class I ADH without involvement of decreased food consumption. These data offer new insights into the toxicity of dioxins *via* a marked decrease in the level of class I ADH.

Key words —— polychlorinated biphenyl, alcohol dehydrogenase, suppression, wasting syndrome, dioxin

INTRODUCTION

3,3′,4,4′,5-Pentachlorobiphenyl (IUPAC PCB number: PCB126) is the most toxic congener of all PCBs and one of the causal agents of Yusho.[1)] The toxic effects of coplanar PCBs have been shown to be similar to those of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and are considered to occur *via* an aromatic hydrocarbon (Ah)-receptor (AhR)-mediated reaction,[2)] although the details are not fully understood. Unlike the induction of several enzymes such as CYP1A1, we know relatively little about the suppression of particular proteins. Current investigations in our laboratory have revealed that protein levels of aldolase B, carbonic anhydrase III, glucose-regulated protein (GRP) 78 (GRP78), GRP94, calreticulin, and calnexin in liver are suppressed by PCB126.[3–6)]

When the cytosolic proteins of PCB126-treated rat liver were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), the intensity of an approximately 40 kDa protein band was markedly low compared to that from free- and pair-fed control groups. After separating the corresponding band in free-fed animals by two-dimensional PAGE and sequencing the peptide fragment obtained by *in situ* digestion with *Staphylococcus aureus* V_8 protease, class I alcohol dehydrogenase (ADH) appeared to be a candidate that is markedly suppressed by dioxins. This study aimed to clarify the significance of ADH suppression by PCB126 using immunoblot analysis.

MATERIALS AND METHODS

PCB126 was synthesized by the method of Saeki *et al*.[7)] The purity of PCB126 was confirmed to be at least 98% by gas chromatography using an electron-capture detector, mass spectroscopy, nuclear magnetic resonance, and UV spectrum.

Male Wistar rats (3 weeks old), purchased from Charles River Japan (Yokohama, Japan), were housed in stainless-steel cages for one week prior to the experiments. The rats were administered PCB126 at a dose of 25 mg/kg/4 ml corn oil i.p. The dose of PCB126 chosen was that known to suppress body weight gain and reduce hepatic aldolase B levels.[3)] Free- and pair-fed animals were treated as described previously.[3)] Each group consisted of 4 rats. Five days after PCB126 administration, the livers were removed and the cytosol fraction prepared by the

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method of Andersson *et al.*[8]

Rabbit antibody was raised to commercial ADH class I (equine liver) (Sigma Chemical Co., St. Louis, MO, U.S.A.) that had been purified to apparent homogeneity by recrystallization according to the method of Andersson *et al.*[8] A male New Zealand White rabbit (2.5 kg body weight) was immunized with an emulsion of the purified alcohol dehydrogenase (100 μg) in physiological saline and BACTO complete Freund's adjuvant (Difco Laboratories, Detroit, MI, U.S.A.). A booster was given two weeks after the initial injection. Blood was collected one week after the booster, and serum was obtained.

SDS-PAGE was performed according to the method of Laemmli.[9] Cytosolic proteins were separated by SDS-PAGE, and then the proteins in the gel were transferred to a polyvinyliden difluoride (PVDF) membrane (Immobilon™ P, Millipore, Bedford, MA, U.S.A.) by the method of Towbin *et al.*[10] with slight modifications as described previously.[3] Anti-ADH antibody produced as described above was used as the primary antibody. Immunochemical visualization was performed according to the method of Guengerich *et al.*[11] with some modifications as described previously.[3] Estimation of the band intensity immunoreactive to anti-ADH was performed using a GT-7600U scanner (Epson, Tokyo, Japan) and NIH image software (version 1.61, Wayrve Rasband, Bethesda, MD, U.S.A.). The immunoblotted membrane was photographed, and a digital image was constructed using a scanner. The scan data were saved and imported into the NIH image software for subsequent analysis.

Protein was assayed by the method of Lowry *et al.*[12] with bovine serum albumin as a standard.

RESULTS

Rats that received the dioxin-like toxic compound PCB126 had marked suppression of body weight gain, corresponding to the so-called wasting syndrome.[13] In addition, liver hypertrophy and atrophy of the spleen and thymus, which are common markers of dioxin toxicity, were also observed (data not shown). These results suggest that the rats developed PCB126 toxicity. The protein level of class I ADH was examined by immunoblotting (Fig. 1). The anti-ADH antibody raised against horse liver class I ADH recognized a single protein with molecular weight of approximately 40 kDa in rat liver cytosol. The molecular weight of the band corresponds well to that reported for rat liver class I ADH.[14] This suggests that the anti-ADH antibody prepared in this study is useful to examine the class I ADH level in rat liver. Figure 1 shows that the band recognized by the anti-ADH antibody was dramatically reduced by PCB126 administration, whereas the levels in free- and pair-fed groups were consistent. PCB126 caused marked suppression of class I ADH from free- and pair-fed controls (Figs. 1 and 2). This result strongly suggests that the reduced class I ADH level is due to PCB126 administration. Restriction of food intake may not be the cause of the marked suppression observed in the PCB126-treated group because pair-feeding showed only a slight effect on the class I ADH level.

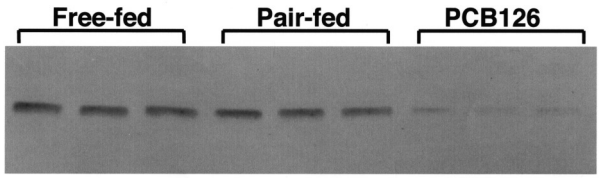

Fig. 1. Immunoblot Analysis of Rat Liver Cytosol Using anti-ADH Antibody

Liver cytosolic protein (3 μg) from free-fed control, pair-fed control, and PCB126-treated rats was subjected to SDS-PAGE (10%). Three samples were randomly selected from four individuals in each group. The primary antibody solution consisted of 1% rabbit anti-horse ADH antiserum, 0.5% normal goat serum, 0.5% bovine serum albumin, 3% skimmed milk, 0.1% sodium azide, and 0.05% Triton X-100 in Tris-buffered saline. Details are described in the text.

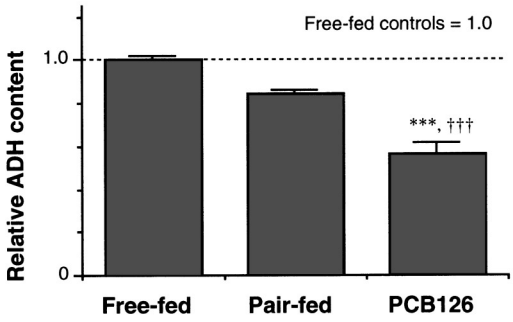

Fig. 2. Ability of PCB126 to Reduce Hepatic ADH in Rats

The ADH content is estimated from Fig. 1 as described in Materials and Methods. The content relative to that of the free-fed control group is shown (free-fed controls = 1.0). The significance was calculated by one-way ANOVA with Scheffe's PLSD. The values represent means ± S.E. ***Significantly different from free-fed controls ($p < 0.001$); †††significantly different from pair-fed controls ($p < 0.001$).

DISCUSSION

We demonstrated for the first time in this study that the ADH class I protein level was significantly

reduced by PCB126 (Figs. 1 and 2). Class I ADH is a dimeric protein found predominantly in liver.[15] Rats have only one class I ADH,[14] whereas class I ADHs in humans are composed of homo- and heterodimers of three very closely related subunits, α, β, and γ.[15]

AhR is believed to be involved in many symptoms caused by dioxins.[2] The involvement of AhR in the regulation of class I ADH is superficial, although the transcriptional regulation of the ADH gene has been well characterized.[16] The importance of CCAAT-enhancer binding protein-α (C/EBPα) on the constitutive expression of class I ADHs is consistent from rats to humans.[16,17] Interestingly, C/EBPα expression is suppressed by TCDD treatment.[18] Taking these results together, class I ADH may be down-regulated through the suppression of C/EBPα by PCB126, although the effect of PCB126 on mRNA levels has not yet been investigated. AhR is possibly involved in the down-regulation of a specific gene(s),[19] although we know relatively little about the mechanism(s) of gene suppression by dioxins. Further investigations are needed to clarify the involvement of AhR in reduced class I ADH protein levels by PCB126.

This study demonstrated that PCB126 suppresses class I ADH, which is a major isoform in rat liver and has the lowest km value for ethanol dehydrogenation among all isoforms.[20] However, there is room for further investigation of the involvement of such environmental pollutants as negative factors in ethanol metabolism.

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REFERENCES

1) Yoshimura, H., Yoshihara, S., Ozawa, N. and Miki, M. (1979) Possible correlation between induction modes of hepatic enzymes by PCBs and their toxicity in rats. *Ann. N.Y. Acad. Sci.*, **320**, 179–192.
2) Poland, A. and Knutson, J. C. (1982) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. *Annu. Rev. Pharmacol. Toxicol.*, **22**, 517–554.
3) Ishii, Y., Kato, H., Hatsumura, M., Ishida, T., Ariyoshi, N. and Oguri, K. (1997) Significant suppression of rat liver aldolase B by a toxic coplanar polychlorinated biphenyl, 3,3′,4,4′,5-pentachlorobiphenyl. *Toxicology*, **116**, 193–199.
4) Ikeda, M., Ishii, Y., Kato, H., Akazawa, D., Hatsumura, M., Ishida, T., Matsusue, K., Yamada, H. and Oguri, K. (2000) Suppression of carbonic anhydrase III in rat liver by a dioxin-related toxic compound, coplanar polychlorinated biphenyl, 3,3′,4,4′,5-pentachlorobiphenyl. *Arch. Biochem. Biophys.*, **380**, 159–164.
5) Tasaki, K., Ishii, Y., Ishida, T. and Oguri, K. (1999) Suppression of stress proteins in endoplasmic reticulum in liver of rats treated with a highly toxic coplanar PCB. *Fukuoka Igaku Zasshi*, **90**, 251–258 (Japanese).
6) Yoshioka, Y., Ishii, Y., Ishida, T., Yamada, H., Oguri, K. and Motojima, K. (2001) Suppression of stress proteins, GRP78, GRP94, calreticulin, calnexin in liver endoplasmic reticulum of rat treated with a highly toxic coplanar PCB. *Fukuoka Igaku Zasshi*, **92**, 201–216 (Japanese).
7) Saeki, S., Yoshihara, S., Uchino, S. and Yoshimura, H. (1979) Improved method of the synthesis of 3,4,5,3′,4′-pentachlorobiphenyl. *Fukuoka Igaku Zasshi*, **70**, 85–87 (Japanese).
8) Andersson, I., Zeppezauer, M., Bull, T., Einarsson, R., Norne, J. E. and Lindman, B. (1979) Binding of coenzyme, coenzyme fragments, and inhibitors to native and carboxymethylated horse liver alcohol dehydrogenase from chlorine-35 nuclear magnetic resonance quadrupole relaxation. *Biochemistry*, **18**, 3407–3413.
9) Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* (London), **227**, 680–685.
10) Towbin, H., Staehelin, T. and Gordon, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. U.S.A.*, **76**, 4350–4354.
11) Guengerich, F. P., Wang, P. and Davidson, N. K. (1982) Estimation of isozymes of microsomal cytochrome P-450 in rats, rabbits, and humans using immunochemical staining coupled with sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Biochemistry*, **21**, 1698–1706.
12) Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randoll, R. J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.
13) Oguri, K., Hatsumura, M., Ishii, Y., Koga, Y., Ariyoshi, N. and Yoshimura, H. (1993) Modification of the gluconeogenesis is not involved in the coplanar PCB toxicity in highly sensitive guinea pigs. *Chemosphere*, **27**, 2295–2303.
14) Lad, P. J. and Leffert, H. L. (1983) Rat liver alcohol dehydrogenase. I. Purification and characterization.

Anal. Biochem., **133**, 350–361.
15) Smith, M., Hopkinson, D. A. and Harris, H. (1971) Developmental changes and polymorphism in human alcohol dehydrogenase. *Ann. Hum. Genet.*, **34**, 251–271.
16) Potter, J. J., Mezey, E., Cornelius, P., Crabb, D. W. and Yang, V. W. (1992) The first base pairs of proximal promoter of the rat class I alcohol dehydrogenase gene is bipartite and interacts with multiple DNA-binding proteins. *Arch. Biochem. Biophys.*, **295**, 360–368.
17) Stewart, M. J., Shean, M. L., Paeper, B. W. and Duester, G. (1991) The role of CCAAT/enhancer-binding protein in the differential transcriptional regulation of a family of human liver alcohol dehydrogenase genes. *J. Biol. Chem.*, **266**, 11594–11603.
18) Liu, P. C., Dunlap, D. Y. and Matsumura, F. (1998) Suppression of C/EBPα and induction of C/EBPβ by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in mouse adipose tissue and liver. *Biochem. Pharmacol.*, **55**, 1647–1655.
19) Dong, L., Ma, Q. and Whitlock, J. P., Jr. (1997) Down-regulation of major histocompatibility complex Q^{1b} gene expression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J. Biol. Chem.*, **272**, 29614–29619.
20) Julia, P., Farres, J. and Pares, X. (1987) Characterization of three isoenzymes of rat alcohol dehydrogenase. Tissue distribution and physical and enzymatic properties. *Eur. J. Biochem.*, **162**, 179–189.