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Effects of Clofibrate on Hyperlipidemia Induced by Polychlorinated Biphenyls and Elimination of Polychlorinated Biphenyls Accumulated in Rat Tissues

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When rats were orally given olive oil for 4 d following the administration of polychlorinated biphenyls (PCBs, KC-400) for 4 d, the amounts of PCBs in liver and adipose tissue were 62.16 and 931.6 μg/g tissue, respectively, while when rats were orally given clofibrate (CPIB) dissolved in olive oil instead of olive oil alone for 4 d following the administration of PCBs for 4 d, the amounts of PCBs in liver and adipose tissue were effectively decreased to 33.70 and 448.8 μg/g tissue, respectively. In addition, when, after oral administration of PCBs for 4 d, rats were maintained by feeding a commercial diet for 14 d and then were orally given olive oil for 4 d, the amounts of PCBs in liver and adipose tissue were 7.70 and 201.4 μg/g tissue, respectively. However, when rats were orally given CPIB dissolved in olive oil instead of olive oil alone for 4 d in the same experimental system, the amount of PCBs (119.8 μg/g tissue) in adipose tissue was remarkably decreased, but that (8.10 μg/g tissue) in liver was unchanged. Further, the increased triglyceride and cholesterol contents in liver and serum of rats that had ingested PCBs were also decreased to the control levels or less by the administration of CPIB.

These results suggest that the residual PCBs in the animal body can be effectively eliminated by CPIB treatment and also that hyperlipidemia and fatty liver induced by PCBs can be normalized by CPIB treatment.

Keywords——polychlorinated biphenyl; clofibrate; hyperlipidemia; fatty liver; liver; adipose tissue

Introduction

Polychlorinated biphenyls (PCBs) tend to accumulate in the food chain and once ingested are mainly stored in the body's fatty tissue, because PCBs in the animal body are not easily excreted or metabolized.[2] Also, PCBs are inducers of hyperlipidemia and hepatic monooxygenase acting on a variety of substrates, including steroids, drugs and polycyclic hydrocarbon carcinogens.[3-5] On the other hand, clofibrate (CPIB) is a hypolipidemic agent commonly used in clinical practice.[6,7] Recently, we have reported that the increase of serum and hepatic cholesterol levels in rats treated with PCBs (KC-400) is depressed by simultaneous administration of CPIB.[8]

In this paper, we investigated the effect of CPIB on the hyperlipidemia induced by PCBs (KC-400) and on the elimination of PCBs accumulated in liver and adipose tissue of rats pretreated with PCBs (KC-400).

Materials and Methods

Chemicals——PCBs (KC-400) were a gift from Dr. Uchiyama (National Institute of Hydienic Sciences, Tokyo, Japan). CPIB was purchased from Tokyo Chemical Ind. (Tokyo, Japan). The other chemicals used here were of reagent grade, purchased from Kanto Chemical Co. (Tokyo, Japan).

Animals——Male albino rats of the Wistar strain, weighing 180—200 g, were used. The rats were fed on a commercial diet obtained from Oriental Yeast Co. (Tokyo, Japan). Food was removed from the cages of all rats at 9:00 am and returned at 6:00 pm. To test the effects of CPIB, PCBs, and PCBs–CPIB, rats were divided into eight

groups of three rats each and treated with the drugs as follows;

Group I (control): Rats were orally given 0.5 ml of olive oil once (9 : 00 am) a day for 4 d and then were orally given 0.5 ml of olive oil three times (9 : 00 am, 3 : 00 pm, 9 : 00 pm) a day for 4 d.

Group II (CPIB): Rats were orally given olive oil as described in group I and then were orally given CPIB (100 mg/kg body) dissolved in 0.5 ml of olive oil three times a day for 4 d as described in group I.

Group III (PCBs): Rats were orally given PCBs (100 mg/kg body) dissolved in 0.5 ml of olive oil once (9 : 00 am) a day for 4 d and then orally given olive oil as described in group I.

Group IV (PCBs–CPIB): Rats were orally given PCBs as described in group III and then were orally given CPIB dissolved in olive oil as described in group II.

Group V (control): After oral administration of 0.5 ml of olive oil once (9 : 00 am) a day for 4 d rats were maintained by feeding a commercial diet for 14 d and then were orally given 0.5 ml of olive oil three times (9 : 00am, 3 : 00pm, 9 : 00pm) a day for 4 d.

Group VI (CPIB): After oral administration of olive oil as described in group V, rats were maintained by feeding a commercial diet for 14 d and then were orally given CPIB (100 mg/kg body) dissolved in 0.5 ml of olive oil three times a day for 4 d as described in group V.

Group VII (PCBs): After oral administration of PCBs (100 mg/kg body) dissolved in 0.5 ml of olive oil once (9 : 00 am) a day for 4 d, rats were maintained by feeding a commercial diet for 14 d and then were orally given olive oil as described in group V.

Group VIII (PCBs–CPIB): After oral administration of PCBs as described in group VII, rats were maintained by feeding a commercial diet for 14 d and then were orally given CPIB dissolved in olive oil as described in group VI.

At 12 h after the final administration of the drugs, blood, liver and adipose tissue (epididymal fat pads) were removed.

Extraction and Determination of PCBs——The extraction of PCBs from 1.5 g of liver or adipose tissue of rats and the clean-up of the extract by column chromatography were performed by the procedure described by Takeshita *et al.*[9] The sample eluted from the silicic acid column with *n*-hexane was concentrated to 10 ml and subjected to gas chromatography. The instrument used for gas chromatography was a Shimadzu GC-7AG gas chromatograph equipped with an electron capture detector. The column (2 m × 3 mm) packing was 2% silicon OV-1 Chromosorb WAW DMCS (80—100 mesh) and the column temperature was maintained at 195 °C. Nitrogen was used as a carrier gas at the flow rate of 80 ml/min. The amount of PCBs was calculated from the sum of the heights of all peaks on a gas chromatogram of residual and standard PCBs (KC-400). The recovery of PCBs was approximately 97.7% under the present experimental conditions.

Determination of Triglyceride, Cholesterol and Phospholipid Contents——Blood was drawn from rats by cardiac puncture into syringe. The liver was immediately perfused with 0.9% of NaCl solution and then homogenized with 2.5—3 volumes of cold 0.01 M phosphate buffer (pH 7.4) containing 1.15% KCl. Serum was obtained from the blood by centrifugation as described previously.[10] The extraction of total lipid in serum and liver homogenates was performed by the procedure of Folch *et al.*[11] For determination of triglyceride and phospholipid, triglyceride and phospholipid in total lipids obtained were separated by silicic acid column chromatography according to the procedure of Sardesai and Manning.[12] After the separation, triglyceride and phospholipid contents were measured by the procedures of Van'Handel and Zilversmit,[13] and Nakagawa and Nishida,[14] respectively. The determination of total cholesterol content was performed as described previously.[8]

Results and Discussion

We first investigated the effect of CPIB on the elimination of PCBs in liver and adipose tissue of rats that had ingested PCBs (KC-400). As shown in Table I, when rats (group III) were orally given olive oil for 4 d following the administration of PCBs for 4 d, the amounts of PCBs in liver and adipose tissue were 62.16 and 931.6 μg/g tissue, respectively, while when rats (group IV) were orally given CPIB dissolved in olive oil instead of olive oil alone for 4 d following the administration of PCBs as described above, the amounts of PCBs in liver and adipose tissue were effectively decreased to 33.70 and 448.8 μg/g tissue, respectively. Similarly, when, after oral administration of PCBs for 4 d, rats (group VII) were maintained by feeding a commercial diet for 14 d and then were orally given olive oil for 4 d, the amounts of PCBs in liver and adipose tissue were 7.70 and 201.4 μg/g tissue, respectively. However, when rats (group VIII) were orally given CPIB dissolved in olive oil instead of olive oil alone for 4 d in the same experimental system, the amounts of PCBs in liver and adipose tissue were 8.10 and 119.8 μg/g tissue, respectively. Accordingly, upon administration of CPIB, the amount of PCBs in adipose tissue was remarkably decreased, while that in the liver was unchanged under

TABLE I. Stimulatory Action of Clofibrate on the Elimination of PCBs Accumulated in Rat Liver and Adipose Tissue

Treatment	Expt. No.	PCB content (μg/g tissue)	
		Liver	Adipose tissue
PCBs	I	56.73±6.53[a]	969.0±106.7[a]
(Group III)	II	67.59±2.43	894.2± 53.2
	Mean±S.D.	62.16±7.33 (100)	931.6± 92.2 (100)
PCBs–CPIB	I	35.30±2.79[a]	498.8± 69.9[a]
(Group IV)	II	32.09±0.46	398.8± 10.7
	Mean±S.D.	33.70±2.55 (54)[b]	448.8± 70.7 (48)[b]
PCBs	I	7.55±0.19	194.6± 3.7
(Group VII)	II	7.85±0.21	208.1± 1.9
	Mean±S.D.	7.70±0.25 (100)	201.4± 7.3 (100)
PCBs–CPIB	I	7.62±0.25	119.1± 1.1
(Group VIII)	II	8.58±0.23	120.8± 0.8
	Mean±S.D.	8.10±0.54 (105)	119.8± 1.2 (59)[b]

Three rats were used for each group in two separate experiments. The values in parentheses are percentages of the values obtained in PCB-treated rats (taken as 100%). *a*) The values are means±S.D. of the values obtained in the assays of each tissue of three rats. The other values are means±S.D. of the values obtained in triplicate assays of pooled tissues of three rats. *b*) Significant at $p<0.001$ by Student's *t*-test.

TABLE II. Effect of Clofibrate on Triglyceride, Cholesterol and Phospholipid Levels in Serum and Liver of Rats Pretreated with PCBs (KC-400)

Treatment	Serum			Liver		
	Triglyceride (μg/ml serum)	Cholesterol (μg/ml serum)	Phospholipid (μg P/ml serum)	Triglyceride (μg/mg protein)	Cholesterol (μg/mg protein)	Phospholipid (μg P/mg protein)
None	457.3± 5.2	754.7±17.2	27.33±3.93	24.23±0.42	9.18±2.21	3.91±0.27
(Group I)	(100)	(100)	(100)	(100)	(100)	(100)
CPIB	296.6±22.9	477.7±22.8	25.79±1.43	16.48±1.21	6.44±0.20	4.06±0.24
(Group II)	(65)[a]	(63)[b]	(94)	(68)[c]	(70)[b]	(104)
PCBs	680.1±20.1	1116.9±25.4	29.70±1.54	41.70±1.04	14.61±0.52	4.98±0.29
(Group III)	(149)[b]	(148)[b]	(109)	(172)[b]	(159)[b]	(127)[a]
PCBs–CPIB	543.5± 3.6	935.8±21.3	28.60±0.54	28.60±0.54	12.75±0.05	5.12±0.07
(Group IV)	(119)[b]	(124)[b]	(104)	(118)[b]	(139)[c]	(131)[c]
None	486.4±15.5	758.0±14.5	28.33±0.63	25.01±1.81	10.91±0.05	4.20±0.11
(Group V)	(100)	(100)	(100)	(100)	(100)	(100)
CPIB	348.2± 6.0	477.7± 2.3	27.20±0.79	15.91±0.49	8.47±0.23	4.17±0.18
(Group VI)	(72)[b]	(63)[c]	(96)	(63)[c]	(78)[b]	(104)
PCBs	585.8±12.4	952.2±20.1	29.39±1.69	45.28±2.11	14.06±0.23	4.15±0.17
(Group VII)	(120)[c]	(126)[b]	(104)	(181)[b]	(129)[b]	(103)
PCBs–CPIB	379.6±13.3	719.4±20.1	27.83±0.24	31.00±2.04	12.96±0.19	4.28±0.30
(Group VIII)	(78)[c]	(95)	(98)	(124)[d]	(119)[b]	(106)

Three rats were used for each group in two separate experiments. The values are means±S.D. of triplicate assays in each experiment. The values in parentheses are percentages of the values obtained in control (none) rats (taken as 100%). *a*) Significant at $p<0.02$ by Student's *t*-test. *b*) Significant at $p<0.001$ by Student's *t*-test. *c*) Significant at $p<0.01$ by Student's *t*-test. *d*) Significant at $p<0.05$ by Student's *t*-test. Triglyceride and phospholipid contents are expressed as tripalmitin and phospholipid phosphorus, respectively.

the present experimental conditions.

KC-400 is a complex mixture consisting mainly of tetrachlorobiphenyls, with tri-, penta- and hexachlorobiphenyls as minor components.[15] The excretion of PCBs is minimal prior to

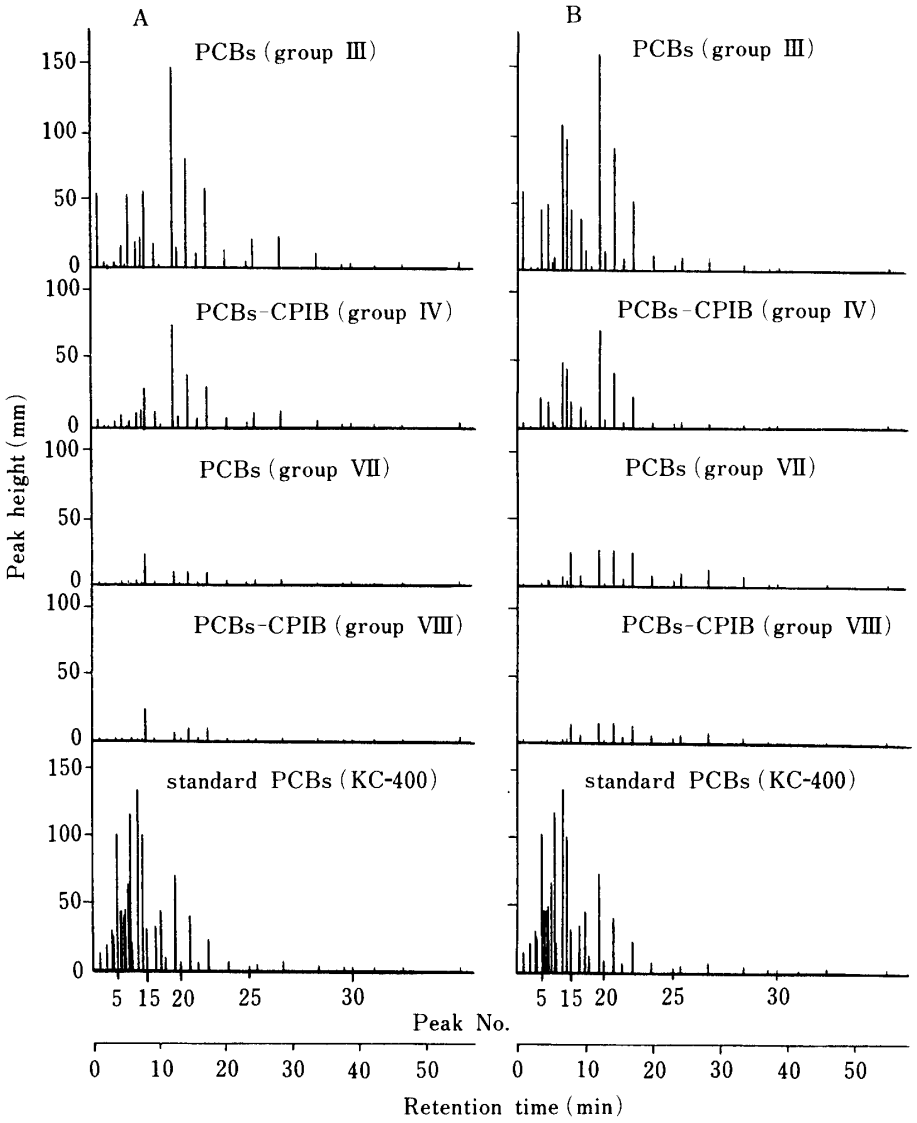

Fig. 1. Peak Pattern in Gas Chromatography of PCBs Obtained from Liver (A) and Adipose Tissue (B) of Rats Treated with PCBs, and PCBs–CPIB

Standard PCBs (KC-400) were injected (35 ng; 5 μl of *n*-hexane solution) into the gas chromatograph. PCB samples extracted from liver and adipose tissue were also injected (2 and 0.2 μl, respectively) into gas chromatograph.

metabolism to more polar components.[2] The metabolism of PCBs is achieved primarily by hepatic mixed-function oxidases and is strongly directed by the position of chlorination.[2] As shown in Fig. 1, there were approximately 33 peaks on a gas chromatogram of standard PCBs (KC-400) under the present experimental conditions. However, the peak pattern on gas chromatograms of residual PCBs in liver and adipose tissue was different from that of standard PCBs. This discrepancy may be due to differences of metabolism of individual PCBs by hepatic mixed-function oxidases rather than differences of absorption and excretion of PCBs. In fact, we have recently reported that the amount of cytochrome P-450 and the activity of drug-metabolizing enzymes in liver microsomes of rats treated with PCBs (KC-400) are further increased by simultaneous administration of CPIB.[8] In addition, Tamburini *et al.*[16] and Yoshimura *et al.*[17] have reported that CPIB and PCBs (KC-400) induce cytochrome P-452 and P-448, respectively. We have confirmed their observations (data not shown). Therefore, PCBs may be effectively metabolized to more polar compounds by cytochrome P-452 induced by CPIB. In any event, the present results suggest that PCBs

accumulated in the animal body may be effectively eliminated by the administration of CPIB.

On the other hand, it is known that triglyceride and cholesterol levels in serum and liver of rats that have ingested PCBs are increased.[18] We have also reported that the increase of hepatic and serum cholesterol contents induced by oral ingestion of PCBs is depressed by simultaneous administration of CPIB[8] and that the hypercholesterolemic effect of CPIB is partly due to the elevation of hepatic mixed-function oxidase activity for cholesterol metabolism.[8] Thus, we next investigated the effect of CPIB on triglyceride, cholesterol and phospholipid levels in serum and liver of rats pretreated with PCBs (KC-400). As shown in Table II, when rats (group III) were orally given olive oil for 4 d following the administration of PCBs for 4 d, triglyceride and cholesterol levels in serum and liver were increased to 149% and 148%, and 172% and 159% of control levels (group I), respectively. However, when rats (group IV) were orally given CPIB for 4 d, triglyceride and cholesterol levels in serum and liver were decreased to 119% and 124%, and 118% and 139% of control levels (group I), respectively. Furthermore, although serum phospholipid levels were unchanged by the administration of CPIB (group II), PCBs (group III), and PCBs–CPIB (group IV), hepatic phospholipid levels were increased by the administration of PCBs (group III), and PCBs–CPIB (group IV). In addition, we obtained similar results when rats that had ingested PCBs were maintained by feeding a commercial diet and then were orally given olive oil with (group VIII) or without (group VII) CPIB. Therefore, hyperlipidemia and fatty liver induced by PCBs (KC-400) may be normalized by the administration of CPIB. Experiments are in progress to study the mechanism of the normalizing effect of CPIB on hyperlipidemia and fatty liver induced by PCBs.

References and Notes

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2) H. B. Mathews and R. L. Dedric, *Ann. Rev. Pharmacol. Toxicol.*, **24**, 85 (1984).
3) J. L. Lincer and D. B. Reakall, *Nature* (London), **228**, 783 (1970).
4) A. P. Alvares, D. R. Bichers and A. Kappas, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 1321 (1973).
5) H. Vainio, *Chem.-Biol. Interact.*, **9**, 379 (1974).
6) N. B. Myant, *The Practioner*, **211**, 188 (1973).
7) M. F. Oliver, *Lancet*, i, 1321 (1962).
8) M. Nakagawa, T. Shimokawa, A. Noguchi, N. Ishihara and S. Kojima, *Lipids*, **21**, 159 (1986).
9) R. Takeshita, M. Suzuki and M. Hayashi, *J. Hygienic Chem.*, **20**, 256 (1974).
10) M. Nakagawa and M. Uchiyama, *Biochem. Pharmacol.*, **23**, 1641 (1974).
11) J. Folch, M. Less and G. H. Sloane-Stanley, *J. Biol. Chem.*, **226**, 497 (1967).
12) V. M. Sardesai and J. A. Mannig, *Clin. Chem.*, **14**, 156 (1968).
13) E. Van Handel and D. B. Zilversmit, *J. Clin. Med.*, **50**, 152 (1973).
14) M. Nakagawa and T. Nishida, *Biochim. Biophys. Acta*, **296**, 577 (1973).
15) S. Saeki, A. Tsutsui, K. Oguri, H. Yoshimura and M. Hamada, *Fukuoka Acta Med.*, **62**, 20 (1971).
16) P. P. Tamburini, H. A. Masson, S. K. Bains, R. J. Makowski, B. Morris and G. G. Gibson, *Eur. J. Biochem.*, **139**, 235 (1984).
17) H. Yoshimura, N. Ozawa and S. Saeki, *Chem. Pharm. Bull.*, **26**, 1215 (1978).
18) N. Nagai, K. Higuchi and Y. Yae, *Fukuoka Acta Med.*, **63**, 367 (1972).