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Comparison of Suppression of Mutagenicity of Benzo(a)pyrene among Methylsulfonyl Polychlorinated Biphenyl Isomers

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Methylsulfonyl (MSF) derivatives of polychlorinated biphenyls (PCBs) were first identified in fat from seals in the Baltic (Jensen and Jansson, 1976). Since then, a number of these substances have been demonstrated in animals (Bergman et al., 1979; Mizutani et al., 1978). They were also isolated from the excreta of mice and rats treated with tri-, tetra-, penta- or hexachlorobiphenyls (Mio et al., 1976). Studies on the metabolic fates of several structurally defined chlorobiphenyls in mice showed that, in addition to the hydroxy species that were considered to be major metabolites of PCBs (Hutzinger et al., 1972), sulfur-containing compounds were formed by the mercapturic acid pathway from PCB arene oxide (Bakke et al., 1983; Preston et al., 1984). The accumulations of some MSF-PCB isomers have been evidenced not only in the mice experimentally ingested with certain PCBs (Brandt et al., 1982) but also in a human being accidentally exposed to PCBs (Yoshida et al., 1979). Even healthy people were found to have MSF-PCB isomers at concentrations as high as those of PCBs (Haraguchi et al., 1986). It is noteworthy that some MSF-PCB isomers have been demonstrated to be toxic for rats and mice (Lund et al., 1986). Moreover, our preliminary study indicated that some MSF-PCB isomers have an inhibitory potency against the aryl hydrocarbon hydroxylase (AHH) activity, a well-known drug-metabolizing enzyme, in cultured human lymphoblastoid cells (Kiyohara et al., 1990). These effects of MSF-PCB isomers seemed comparable to those of the well known 7,8-benzoflavone (7,8-BF), which inhibits chemical carcinogenesis (Wattenberg et al., 1977; Wiebel, 1980).

In the present study, we studied the effect of 11 MSF-PCB isomers and 7,8-BF on the mutagenicity of benzo(a)pyrene (BP) using Salmonella strains TA98 and TA100 in the Ames assay. In addition, the relationship between the results of Ames assay and the AHH assay was investigated.

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MATERIALS AND METHODS

The 11 MSF-PCB isomers used in this study are shown in Table 1. The MSF-PCB isomers as well as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were synthesized and purified as previously described (Haraguchi et al., 1987). The purities of these compounds were more than 99% when analyzed by gas chromatography. 7,8-BF and BP were obtained from Wako Pure Chemical Ind., Ltd., Osaka, Japan and recrystallized from ethanol and methanol, respectively.

Lymphoblastoid cells were cultured in RPMI-1640 medium supplemented with 20% heat-inactivated fetal bovine serum (FBS), penicillin (100 units/ml) and streptomycin (100 μg/ml). The cells were seeded at a density of approximately 3 x 10^5 cells/ml and the cultures were grown at 37°C in an atmosphere of fully humidified air with 5% CO_2. The AHH activity was induced by adding either 3-methylcholanthrene (MC; 0.7 μg/ml) or TCDD (3 ng/ml) dissolved in acetone to the culture medium, which was then kept for 48 hrs (to obtain the MC-induced and TCDD-induced AHH activity). A control culture (to measure control AHH activity) was added with acetone alone (5 μl/ml) at the same time schedule. For the inhibition of enzyme, the cultured cells were treated with any of the 11 MSF-PCB isomers (1.5 μg/ml culture medium) or 7,8-BF (1.4 μg/ml culture medium) and cultured for an additional 48 hrs. The control cells received solvent acetone alone again at this time. The cells from the culture flasks with a viability of 90% or more were harvested, washed twice with 0.05 M Tris-HCl buffer (pH 8.5) supplemented with 0.2 M sucrose and 3 mM $MgCl_2$ and assayed for the AHH activity at 37°C for 50 min with BP as a substrate (Kiyohara et al., 1990).

The mutagenicity tests were conducted according to Maron and Ames (1983), using tester strain TA98 and TA100 (supplied by Dr. B.N. Ames, University of California, Berkeley, U.S.A.). The S-9 was prepared from male Wistar-King (250-300 g) rats pretreated with polychlorinated biphenyls, Kanechlor 500 (Kanegafuchi Kagaku, Osaka, Japan), and used for the enzyme source for metabolic activation. MSF-PCB isomers (3.0 mg/ml), 7,8-BF (2.8 mg/ml) and BP (0.1 μg/ml) were dissolved in acetone. Acetone (50 μl/plate) was used as a negative control. Each samples was plated in duplicate.

RESULTS AND DISCUSSION

Table 1 shows the effects of 11 MSF-PCB isomers and 7,8-BF on AHH activities in human lymphoblastoid cells. The AHH induction by MC or TCDD were 3.93 and 5.83, respectively. The AHH activities (control, MC-induced or TCDD-induced AHH activities) were assayed after the cells were incubated with 7,8-BF or with the 11 MSF-PCB isomers individually or the solvent acetone alone. Eight of the isomers markedly inhibited the control activity (see the column control). In particular 4-MSF-3,3',4',5-tetraCB and the 3-MSF-3',4,4',5-tetraCB strongly

Table 1. Effect of MSF-PCB isomers on control, MC-induced and TCDD-induced AHH activities in human lymphoblastoid cells

Chemical	Inhibition rate[a] (%)		
	Control	MC-induced	TCDD-induced
Acetone	0 (0.029)[b] 1.0[c]	0 (0.114) 3.93[c]	0 (0.169) 5.83[c]
7,8-BF	74	80	95
Tetrachlorobiphenyl			
3-MSF-2,3',4',5-	24	0	16
3-MSF-2',3',4,5-	33	40	37
3-MSF-3',4,4',5-	69	18	79
4-MSF-2,2',5,5'-	-3	6	31
4-MSF-3,3',4',5-	68	46	82
Pentachlorobiphenyl			
3-MSF-2',3',4,4',5-	5	15	34
3-MSF-3',4,4',5,5'-	-94	29	11
4-MSF-2,2',3',4',5-	19	-3	34
4-MSF-3,3',4',5,5'-	42	45	67
Hexachlorobiphenyl			
3-MSF-2',3',4,4',5,5'-	6	4	34
Heptachlorobiphenyl			
3-MSF-2',3',4,4',5,5',6-	-2	14	-20

a: Inhibition rate (%)=[1-(any of 11 MSF-PCB isomers or 7,8-BF-treated AHH activity/acetone-treated AHH activity)*100

b: Values in parenthesis represent AHH activity expressed in terms of 3-hydroxy-BP (pmoles/min/10^6 cells) formed.

c: Values represent AHH inducibility (MC- or TCDD-induced AHH activity/control AHH activity).

inhibited control AHH activity (close to 70%). 7,8-BF inhibited the control AHH activity by 74%. On the contrary, 3-MSF-3',4,4',5,5'-pentaCB increased the control AHH activity by 94%. The effects on the MC-induced AHH activity are summarized in the column MC-induced. The largest inhibition was produced by 4-MSF-3,3',4',5-tetraCB, followed by 4-MSF-3,3',4',5,5'-pentaCB and 3-MSF-2',3',4,5-tetraCB (40 to 46%). 7,8-BF decreased the MC-induced activity by 80%, a similar value to that for the control AHH. The TCDD-induced AHH activity (the column TCDD-induced) was influenced most among the three AHH activities. Almost all MSF-PCB isomers examined, particularly 4-MSF-3,3',4',5-tetraCB, 3-MSF-3',4,4',5-tetraCB and 4-MSF-3,3',4',5,5'-pentaCB, effectively decreased the enzyme activity (67% to 82%). Also

Table 2. Comparison of suppression of mutagenicity of BP among MSF-PCB isomers or 7,8-BF in Ames Salmonella/microsome assay

Test system[a] (BP plus)	No. of Revertants		Suppression rate[b] (%)	
	TA98	TA100	TA98	TA100
7,8-BF	105	177	80	79
Tetrachlorobiphenyl				
3-MSF-2,3',4',5-	309	733	40	14
3-MSF-2',3',4,5-	359	683	31	20
3-MSF-3',4,4',5-	177	333	66	61
4-MSF-2,2',5,5'-	706	923	-36	-8
4-MSF-3,3',4',5-	259	553	50	35
Pentachlorobiphenyl				
3-MSF-2',3',4,4',5-	550	797	-6	7
3-MSF-3',4,4',5,5'-	218	457	58	46
4-MSF-2,2',3',4',5-	547	775	-6	9
4-MSF-3,3',4',5,5'-	172	379	67	56
Hexachlorobiphenyl				
3-MSF-2',3',4,4',5,5'-	434	711	16	17
Heptachlorobiphenyl				
3-MSF-2',3',4,4',5,5',6-	322	691	38	19

a:The number of revertants per plate induced by BP (5 μg/plate) alone was 519 for TA98 and 854 for TA100.
b:Suppression rate (%)=[1-(number of revertants induced by BP with the test compound)/(number of revertants induced by BP without the test compound)]*100.

7,8-BF strongly reduced the TCDD-induced AHH activity (by 95%). Thus among the isomers of MSF-PCB, 3-MSF-3',4,4',5-tetraCB was the most marked in inhibitory potency particularly to the control and TCDD-induced AHH activities, although the extent of inhibition was smaller than that by 7,8-BF.

The inhibitory spectrum of MSF-PCB isomers on the TCDD-induced AHH activity resembled those on the control AHH activity but were somewhat different from those on the MC-induced AHH activity. These findings imply that cytochrome P-450 isozymes which are present constitutively are qualitatively similar to TCDD-induced P-450 isozymes but not different from the MC-induced P-450 isozymes in human lymphoblastoid cells.

Table 2 shows the suppression of mutagenicity of BP by 11 MSF-PCB isomers or 7,8-BF. TCDD, the most potent AHH inducer, was not mutagenic in the Ames assay using Salmonella typhimurium TA98 and TA100 (Kiyohara et al., in press), while 7,8-BF strongly suppressed the mutagenic activity of BP (80 %).

Table 3. Correlation coefficient between inhibition of AHH activity and suppression of mutagenicity of BP

	Control AHH activity	MC-induced AHH activity	TCDD-induced AHH activity
TA98	0.413[a] (p=0.183)	0.683 (p=0.014)	0.476 (p=0.118)
TA100	0.345[a] (p=0.271)	0.766 (p=0.003)	0.640 (p=0.025)

a:The exclusion of 3-MSF-3',4,4',5,5'-pentaCB from data analysis of 12 chemicals (11 MSF-PCB isomers and 7,8-BF) results in statistical significance with correlation coefficient (R=0.807, p=0.0027 for TA98 and R=0.866, p=0.0006 for TA100).

Eight of 11 MSF-PCB isomers suppressed the mutagenicity of BP, with 3-MSF-3',4,4',5-tetraCB and 4-MSF-3,3',4',5,5'-pentaCB being potent suppressors. 3-MSF-2',3',4,5-tetraCB, 4-MSF-3,3',4',5-tetraCB and 3-MSF-3',4,4',5,5,5'-pentaCB were moderate suppressors, while 4-MSF-2,2',5,5'-tetraCB enhanced the mutagenicity of BP and 3-MSF-2',3',4,4',5-pentaCB produced no effects. These results show that 3-MSF-3',4,4'5-tetraCB and 4-MSF-3,3',4',5,5'-pentaCB have both AHH inhibitory effect and strong suppressing potency on the mutation induced by BP. It, therefore, appears that the suppression of BP by MSF-PCB isomers and 7,8-BF may in part relate to their ability to inhibit cytochrome P-450 dependent metabolic activation of polycyclic aromatic hydrocarbon which in turn results in the inhibition of mutagenicity in Ames Salmonella/microsome assay.

The correlation coefficient between inhibition of AHH activities and suppression of mutagenicity of BP is shown in Table 3. In both strains a statistically significant correlation was seen in the MC- or TCDD-induced AHH activity while not in the control AHH activity. If 3-MSF-3',4,4',5,5'-pentaCB has been excluded from data analysis of 12 chemicals (11 MSF-PCB isomers and 7,8-BF), the observed correlation coefficient in the control AHH activity is statistically significant .

The substitution of either of the 4 or 5 chlorine atoms at the lateral positions of the biphenyl moiety, namely, 3, 3', 4, 4', 5 and 5' positions, seemed necessary for the inhibition of the AHH activity or suppression of mutagenicity of BP. These positional requirement resembles that of the non-ortho substituted coplanar PCBs, namely, 3,3',4,4'-tetraCB, 3,3',4,4',5-pentaCB and 3,3'4,4',5,5'-hexaCB, which are known to be the most toxic congeners of PCBs (Kannan et al., 1988).

The further studies should be conducted on the structure-activity relationships (inhibition of AHH and suppression of

mutagenicity) of additional MSF-PCB isomers and also in other short-term tests (e.g., micro nuclei test, sister chromatid exchange and rec assay).

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