ANALYSIS OF THE TUMOR-PROMOTING POTENCY OF 2,4,4'-TRICHLOROBIPHENYL AND 2,2',4,5,5'-PENTACHLOROBIPHENYL IN RAT LIVER

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Introduction

Polychlorinated biphenyls (PCBs) are potent persistent environmental pollutants exhibiting neurotoxic, teratogenic and tumor-promoting effects in experimental animal models[1,2,3]. PCB congeners can be divided into 'dioxin-like' and 'non-dioxin-like' congeners on the basis of their ability to act as aryl hydrocarbon receptor (AhR) agonists. Like the most toxic dioxin congener 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) 'dioxin-like' PCBs bind to the AhR and show characteristic effects on the expression of AhR-regulated genes including the induction of cytochrome P450 (CYP) 1A1[3]. On the other hand, 'non-dioxin-like' PCB congeners have a lower or no binding affinity to the AhR, but exhibit a 'phenobarbital-type' induction of CYP 2B1/2 activity[3,4]. The tumor-promoting potency of several PCBs has been demonstrated in two-stage initiation-promotion experiments in rat liver[2,5]. Preneoplastic cell clones, targets for tumor promotion, can be identified as phenotypically altered foci showing characteristic enzyme patterns including the decreased activity of adenosine triphosphatase (ATPase) or the increased expression of the placental form of gluthatione S-transferase (GSTP)[6].
In the present study, the effect of the 'non-dioxin-like' 2,4,4'-trichlorobiphenyl (PCB 28) and 2,2',4,5,5'-pentachlorobiphenyl (PCB 101) on the promotion of enzyme-altered hepatic foci was investigated in female Wistar rats after initiation with diethylnitrosamine (DEN).

Materials and Methods

Experimental design. Female Wistar rats weighing 75-100 g were obtained from Charles River (Kisslegg, Germany) and kept under standard conditions. Rats were allowed to adjust for one week and then divided into treatment groups (12 per group) as shown in table 1. For inititation of preneoplastic lesions DEN was administered p.o. as an aqueous solution over 10 days, control

groups received water. Following a recovery period of eight weeks animals were treated weekly with corn oil (controls) or PCBs (dissolved in corn oil) by i.p. injection over 8 or 16 weeks. Bromodeoxyuridine (BrdU) was injected i.p. three times at intervals of four hours at a dose of 30 mg/kg body weight (bw) 16 hours prior to sacrifice. After eight and sixteen weeks, six animals per group were sacrificed by decapitation and livers were removed. Liver sections were frozen on dry ice or fixed in Carnoy solution immediately for immunohistochemical analysis.

Table 1. Treatment groups

Group	Treatment
1	Water / Corn oil
2	DEN [10 mg/kg bw] / Corn oil
3	DEN [10 mg/kg bw] / PCB 28 [150 μmol/kg bw]
4	DEN [10 mg/kg bw] / PCB 28 [50 μmol/kg bw]
5	DEN [10 mg/kg bw] / PCB 101 [150 μmol/kg bw]
6	DEN [10 mg/kg bw] / PCB 101 [50 μmol/kg bw]
7	Water / PCB 28 [150 μmol/kg bw]
8	Water / PCB 101 [150 μmol/kg bw]

Immunostaining and quantitation of preneoplastic liver lesions. Liver sections were prepared on a cryostat microtome and stained for ATPase. Additional sections were stained immunohistochemically against the placental form of GST. Lesions showing either decreased ATPase activity or increased GSTP expression were projected on a digitizer screen and the outlines of lesions were traced with a cursor. The total number of enzyme altered foci per cm^2 liver tissue was calculated (Saltykov and Fullman method)[7,8,9]. In addition to GSTP immunostaining the cells replicating DNA were labeled with a BrdU-antibody.

PCB load. Livers were homogenized with Na_2SO_4 (1 g liver/ 10 g Na_2SO_4) and 10 % of the homogenate was extracted with 10 ml dichloromethane/cyclohexane. Solvent was added to a final volume of 10 ml and half of it was supplemented 1:1000 with a PCB 28/101 standard solution (1 μg/μl). The volume of the sample was reduced to approximately 50 μl and the amount of PCB was analyzed with GC/MS. The amount of fat was quantified by dry weight after extraction of the liver homogenate with the solvent.

Statistical analysis. Data in tables represent means ± standard deviation. Relative liver weights were tested for significant differences with One-way ANOVA and Dunnett's Post test. All other data were compared using Student's t-test. The significance level chosen for all statistical analyses was $p \leq 0.05$.

Results and discussion

Three animals did not survive the treatment period with DEN and PCBs, the general appearance of all other animals in the different treatment groups was normal.

Table 2 shows the relative liver weight of rats after eight weeks. Animals treated with DEN and PCB 28 showed a significant increase in relative liver weight at both doses (150 μmol/kg bw, 50 μmol/kg bw). However, this effect was not seen for rats treated with DEN and PCB 101. PCB levels in rat liver were higher for the congener PCB 28 than for PCB 101, possibly due to the lower

degree of chlorination. The amount of PCBs in liver showed a significant difference for rats treated with the high and low dose of each congener.

Table 2. Relative liver weight and hepatic PCB levels in female Wistar rats after treatment with DEN and PCBs for 8 weeks.

Treatment group	No. of animals	rel. liver weight [%]	PCB 28 [µg/kg liver]	PCB 28 [µg/g fat]	PCB 101 [µg/kg liver]	PCB 101 [µg/g fat]
1	6	2.76 ± 0.15	10.49 ± 2.95	0.20 ± 0.07	28.86 ± 3.89	0.56 ± 0.15
2	5	2.80 ± 0.20	19.50 ± 4.76	0.31 ± 0.09	31.83 ± 6.62	0.49 ± 0.08
3	6	3.29 ± 0.22[a,b]	29611.39 ± 11340.39	565.31 ± 243.07	29.14 ± 4.06	0.56 ± 0.12
4	5	3.19 ± 0.17[a,b]	10469.33 ± 2819.77[c]	217.38 ± 63.41[c]	26.33 ± 3.01	0.56 ± 0.17
5	6	3.04 ± 0.15	28.86 ± 7.82	0.55 ± 0.22	3098.14 ± 687.70	58.51 ± 21.66
6	6	2.89 ± 0.18	30.43 ± 12.82	0.59± 0.23	2062.29 ± 547.25[c]	39.35 ± 11.30[c]
7	6	2.92 ± 0.14	31165.71 ± 7566.14	624.05 ± 146.53	29.14 ± 4.34	0.58 ± 0.10
8	6	3.02 ± 0.29	25.71 ± 13.68	0.52 ± 0.28	2997.43 ± 1615.45	61.51 ± 33.74

[a] significantly different from untreated controls
[b] significantly different from DEN treated animals
[c] significantly different from animals treated with PCB 28 or PCB 101 in high dosis

After 16 weeks of treatment a significant increase in relative liver weight was found for animals treated with DEN and PCB 28 at the high dose (150µmol/kg bw). The PCB levels in rat liver were comparable to those after 8 weeks (table 3). The difference between the two chosen time points is not quite significant. These findings indicate a steady state of PCB levels in rat liver and lack of accumulation.

Table 3. Relative liver weight and hepatic PCB levels in female Wistar rats after treatment with DEN and PCBs for 16 weeks.

Treatment group	No. of animals	rel. liver weight [%]	PCB 28 [µg/kg liver]	PCB 28 [µg/g fat]	PCB 101 [µg/kg liver]	PCB 101 [µg/g fat]
1	6	2.81 ± 0.08	29.14 ± 8.99	0.61 ± 0.20	28.00 ± 5.42	0.59 ± 0.13
2	6	2.97 ± 0.23	24.71 ± 4.64	0.57 ± 0.15	24.86 ± 3.02	0.57 ± 0.13
3	6	3.54 ± 0.46[a,b]	37247.57 ± 8355.96	718.30 ± 211.87	22.14 ± 2.12	0.42 ± 0.07

Table 3. Relative liver weight and hepatic PCB levels in female Wistar rats after treatment with DEN and PCBs for 16 weeks (continued).

Treatment group	No. of animals	rel. liver weight [%]	PCB 28 [µg/kg liver]	PCB 28 [µg/g fat]	PCB 101 [µg/kg liver]	PCB 101 [µg/g fat]
4	5	3.02 ± 0.31	11194.00 ± 2853.11[c]	255.55 ± 41.43[c]	104.17 ± 199.80	2.34 ± 3.16
5	6	2.97 ± 0.17	35.00 ± 21.08	0.81 ± 0.64	2929.43 ± 1201.02	61.97 ± 31.80
6	6	2.76 ± 0.17	16.00 ± 3.42	0.36± 0.07	1461.43 ± 258.92[c]	32.94 ± 6.41[c]
7	6	2.99 ± 0.22	27869.86 ± 9145.68	644.37 ± 203.75	28.14 ± 8.93	0.66 ± 0.20
8	6	2.88 ± 0.19	58.83 ± 94.55	1.13 ± 1.76	3680.71 ± 714.86	71.52 ± 20.13

[a] significantly different from untreated controls
[b] significantly different from DEN treated animals
[c] significantly different from animals treated with PCB 28 of PCB 101 in high dosis

The rate of DNA synthesis, as meaured by the BrdU labeling index of nuclei in preneoplastic foci, was not enhanced significantly at both time points (table 4 and 5). However, the increase in relative liver weight after treatment with DEN/PCB 28 implicates a liver growth by hypertrophy rather than liver cell hyperplasia.

As seen in table 4, administration of PCB 28 or PCB 101 at both doses for 8 weeks did not promote significantly the number of enzyme altered hepatic foci induced by DEN.

Table 4. BrdU labeled nuclei and occurrence of enzyme altered hepatic liver foci after 8 weeks of DEN and PCB treatment.

Treatment group	No. of animals	BrdU labeling nuclei/mm^2	No. of ATPase negative foci/cm^3 liver (Saltykov)	No. of ATPase-negative foci/cm^3 liver (Fullman)	No. of GSTP-positive foci/cm^3 liver (Saltykov)	No. of GSTP-positive foci/cm^3 liver (Fullman)
1	6	0	0	0	0	0
2	5	15.00 ± 28.93	53.60 ± 69.30	52.40 ± 67.70	537.40 ± 439.40	526.80 ± 429.90
3	6	10.24 ± 16.13	82.33 ± 116.76	83.83 ± 117.02	412.00 ± 454.96	401.00 ± 432.00
4	5	4.10 ± 7.03	17.20 ± 21.07	17.40 ± 21.33	322.60 ± 332.34	309.60 ± 327.98
5	6	32.86 ± 33.79	75.00 ± 40.62	75.50 ± 39.69	375.50 ± 420.06	376.00 ± 419.28
6	6	11.08 ± 20.85	49.00 ± 52.27	49.17 ± 52.72	237.00 ± 211.79	230.50 ± 211.71

Table 4. BrdU labeled nuclei and occurrence of enzyme altered hepatic liver foci after 8 weeks of DEN and PCB treatment (continued).

Treatment group	No. of animals	BrdU labeling nuclei/mm^2	No. of ATPase-negative foci/cm^3 liver (Saltykov)	No. of ATPase-negative foci/cm^3 liver (Fullman)	No. of GSTP-positive foci/cm^3 liver (Saltykov)	No. of GSTP-positive foci/cm^3 liver (Fullman)
7	6	0	26.67 ± 39.61	23.50 ± 34.01	58.83 ± 131.56	53.83 ± 120.37
8	6	0	0	0	0	0

A statistically significant increase in the number of ATPase-deficient foci/cm^2 liver was only seen for the high dose of PCB 101 after 16 weeks. The number of GSTP-positive foci in this treatment group, however, did not significantly differ from those in animals treated with DEN only (table 5).

Table 5. BrdU labeled nuclei and occurrence of enzyme altered hepatic liver foci after 16 weeks of DEN and PCB treatment.

Treatment group	No. of animals	BrdU labeling nuclei/mm^2	No. of ATPase-negative foci/cm^3 liver (Saltykov)	No. of ATPase-negative foci/cm^3 liver (Fullman)	No. of GSTP-positive foci/cm^3 liver (Saltykov)	No. of GSTP-positive foci/cm^3 liver (Fullman)
1	6	0	0	0	0	0
2	6	9.11 ± 9.42	34.83 ± 43.28	35.00 ± 43.29	375.67 ± 565.26	381.83 ± 573.16
3	6	15.55 ± 18.72	118.00 ± 108.76	120.17 ± 112.96	301.83 ± 339.33	300.67 ± 345.42
4	5	3.87 ± 8.64	43.40 ± 35.44	43.80 ± 36.65	163.00 ± 115.97	166.20 ± 119.07
5	6	15.34 ± 11.14	63.67 ± 32.34	63.17 ± 32.51	446.83 ± 329.51	450.50 ± 331.05
6	6	6.45 ± 7.81	78.67 ± 26.44[d]	79.17 ± 24.90[d]	216.00 ± 194.85	212.83 ± 194.18
7	6	0	12.50 ± 10.40	12.50 ± 10.42	0	0
8	6	0	0	0	6.67 ± 14.91	6.00 ± 13.42

[d] significantly different from DEN treated animals

Experiments with rat hepatocytes in vitro showed a significant inhibition of UV-induced apoptosis by different tumor-promoting agents including the PCB congeners 28 and 101[10]. In contrast, our findings suggest no significant tumor-promoting effect of PCB 28 and a week promoting effect of PCB 101 in female Wistar rats in vivo after initiation of preneoplastic lesions with DEN in a two-stage initiation-promotion study.

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