The tumor promoting potential of polychlorinated dioxins and biphenyls: mechanisms of action in different in vitro assays.

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INTRODUCTION

Studies into the mechanisms of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced carcinogenesis have suggested that TCDD is an extremely potent tumor promoter in rodent liver. Enhancement of preneoplastic lesions in the rat liver by TCDD is correlated with stimulation of hepatocellular growth[1]. However, the interaction of TCDD with growth factors and cellular signalling pathways in tumor promotion remains unclear. Possibly, the induction of arachidonic acid metabolism[2] and an enhanced production of prostaglandins[3] are involved. It is, therefore, the objective of the present study to establish a battery of in vitro tests for the evaluation of the tumor promoting potential of TCDD and related biphenyls and to investigate the role of arachidonic acid metabolism as a possible mechanism of their promoting activity. Indomethacin, an inhibitor of cyclooxygenase, was found to prevent the promoting effect of TCDD, i.e., the promotion of malignant cell transformation and the stimulation of hepatocellular proliferation. Thus, the results suggest that metabolites of arachidonic acid might be essential for the tumor promotion by TCDD.

MATERIALS AND METHODS

TCDD was a gift from Professor D. Neubert, Institut für Toxikologie und Embryopharmakologie, Freie Universität Berlin (F.R.G.). The biphenyl congeners #77/ 3,3´,4,4´-tetrachlorobiphenyl (TCB) and #153/ 2,2´,3,3´,5,5´-hexachlorobiphenyl (HCB) were obtained from the Ökometric GmbH (Bayreuth, F.R.G.). The test substances had a purity of greater than 99%.

Hepatocytes were isolated by collagenase perfusion from male Wistar rats and cultured as described[4]. On the second day after cell plating, the mitotic index was determined after 3 h exposure to colchicine (0.1 mM).
The transformation assay with C3H/ M2 mouse fibroblasts was adjusted to determine tumor promotion according to procedures described previously[5].
Differentiation of C2 Myf4 CAT cells was measured as chloramphenicol acetyltransferase (CAT) activity which in these cells is driven by a myogenic differentiation promoter (Myf4). C2 Myf4 CAT cells were incubated with horse (5%) or fetal calf (20%) serum for 12 h, then test compounds were added for 48 h. Thereafter, cells were harvested, and CAT activity was assayed in the cytosolic fraction. After incubation with ^{14}C-chloramphenicol the labelled acetylated derivatives were separated chromatographically, detected by autoradiographic analysis and quantified by liquid scintillation counting.

RESULTS

Tumor Promoting Action of TCDD and TCB

The effects of tumor promoters were maximal with TCDD, 10^{-12} M or TCB, 10^{-9} M and decreased at higher concentrations of these agents:
1. TCDD and TCB significantly stimulated the mitotic rates in rat hepatocytes cultured under serum-free conditions with insulin (10^{-8}M), dexamethasone (10^{-9}M) and epidermal growth factor (20 ng/ ml).
2. TCDD and TCB enhanced the rates of malignant transformation of M2 fibroblasts induced by N-methyl-N´-nitro-N-nitrosoguanidine (MNNG) or methylcholanthrene (MCA). Without pretreatment with these genotoxic agents, no transforming effect of TCDD or TCB on fibroblasts was observed.
3. TCDD and TCB induced CAT activity in the newly established cell line C2 Myf4 CAT in which the CAT gene is under the control of a myogenic differentiation promoter (Table 1). HCB, a phenobarbital-like inducer of cytochromes, induced differentiation in these cells only at higher concentrations, i.e., at 10^{-7} M; its effects in the other assays remain to be studied.

Table 1: Differentiation of the C2 Myf4 CAT cell line

Culture	relative CAT activity (cpm%)						
	CON	TCDD(M)				TCB(M)	
		10^{-13}	10^{-12}	10^{-11}	$3x10^{-10}$	$3x10^{-9}$	$3x10^{-8}$
+FCS	0.4	0.4	0.7	0.6	0.6	0.6	0.5
+Horse	7.5	10.9	10.8	8.7	9.5	11.2	7.2

Inhibition of the effects of TCDD and TCB by indomethacin

To investigate the role of arachidonic acid metabolism in the action of TCDD, hydrocortisone (1ug/ ml), an inhibitor of phospholipase A_2, and indomethacin (20 ug/ ml), an inhibitor of cyclooxygenase, were added to the M2 fibroblast transformation assay. 12-O-Tetradecanoylphorbol-13-acetate (TPA) was used as a positive control. In agreement with the results of others[3], the promoting effect of TPA was inhibited by hydrocortisone and indomethacin. The promoting effect of TCDD in this assay was also prevented by these inhibitors (Table 2). Moreover, indomethacin (20 ug/ml) abolished the stimulation of mitosis by TCDD or TCB in rat hepatocytes in vitro.

Table 2: Inhibition of TCDD-induced promotion of C3H/M2 transformation by inhibitors of prostaglandin formation

Treatment	DMSO	MNNG	MCA
DMSO (0.5%)	0/24[a]	1/20	3/22
DMSO + Hydrocortisone	0/24	1/24	0/22
DMSO + Indomethacin	0/19	1/17	2/16
TPA (0.25 ug/ ml)	0/14	5/15	4/13
TPA + Hydrocortisone	0/10	0/13	1/11
TPA + Indomethacin	0/14	1/15	1/14
TCDD (1.5 pM)	0/22	6/21	7/20
TCDD + Hydrocortisone	0/16	1/22	2/22
TCDD + Indomethacin	0/22	1/16	2/22

a)Number of transformed foci per treated dishes

DISCUSSION AND CONCLUSIONS

To investigate the tumor promoting activity of dioxins and related biphenyls in vitro, a battery of different tests was used.

TCDD is known to stimulate growth in the rodent liver in vivo and in vitro. However, the rates of DNA synthesis and mitosis vary markedly in hepatocyte preparations from different rats and the stimulation by tumor promoters is only transient[1,4]. Thus, stimulation of cell proliferation might be an important aspect of tumor promotion, but is not sufficient to explain the promoting effect.

Long-term effects of tumor promoters can be studied in vitro by determining the transformation of fibroblasts after initiation with genotoxic carcinogens. A good correlation was found between the dose-response relationships of TCDD and TCB in the transformation assay and in the hepatocellular growth stimulation assay.

Recently, we added to our test battery another short-term assay, i.e., the differentiation of C2 Myf4 CAT cells. The induction of myogenic differentiation by TCDD and TCB in this cell line was in accord with our earlier findings that TCDD and TCB induced the erythroid differentiation in the mouse leukemia cell line F4-6[6].

Furthermore, we were interested in the underlying mechanism of the effects of TCDD and TCB in the different cell systems. The results form experiments with inhibitors of arachidonic acid metabolism, e.g. indomethacin, suggest that eicosanoids might play an essential role in tumor promotion by TCDD. Thus, different tumor promoters, e.g. TPA and TCDD, which bind to different cellular acceptor molecules might have a common mechanism of action, i.e., the stimulation of arachidonic acid metabolism.

REFERENCES

1 Lucier GW, Tritscher AM, Goldsworthy T, Foley J, Clark G, Goldstein J, Maronpot R. Ovarian hormones enhance TCDD-mediated increases in cell proliferation and preneoplastic foci in a two stage model for hepato-carcinogenesis. Cancer Res. 1991; 51: 1391-97.

2 Rifkind AB, Gannon M, Gross SS. Arachidonic acid metabolism by dioxin-induced cytochrome P-450: a new hypothesis on the role of P-450 in dioxin toxicity. Biochem.Biophys.Res.Commun. 1990; 172: 1180-88.

3 Semba M, Inui N. Inhibition of 12-O-tetradecanoylphorbol-13-acetate-enhanced transformation in vitro by inhibitors of phospholipid metabolism. Toxicol.Lett. 1990; 51: 1-6.

4 Wölfle D, Becker E, Schmutte C. Growth stimulation of primary rat hepato-cytes by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Cell Biol. Toxicol. 1993; 9: 15-31.

5 Wölfle D, Schmutte C, Westendorf J, Marquardt H. Hydroxyanthraquinones as tumor promoters: Enhancement of malignant transformation of C3H mouse fibroblasts and growth stimulation of primary rat hepatocytes. Cancer Res. 1990; 50: 6540-44.

6 Wölfle D, Schaefer A, Marquardt H. Effect of the tumor promoters 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 3,3′,4,4′-tetrachlorobiphenyl (TCB) on leukemia cell differentiation. Proc.Amer.Assoc.Cancer Res. 1990; 31: 155.