Tumor-Promotive and Biochemical Effects of Single-Dose Polychlorinated Biphenyls and Dioxin in Mice

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ABSTRACT

The polychlorinated biphenyls (PCBs) and dioxins, as persistent environmental and tissue contaminants, are of concern with regard to prolonged biochemical effects, including tumor promotion. As a model for such effects, we have followed both tumor promotion and longterm biochemical changes in target organs of mice following single-dose exposure to PCBs and the dioxin, TCDD. A single dose of Aroclor 1254 (250 mg/kg) on postnatal day 8 to male Swiss mice promoted the development of both liver and lung tumors initiated by N-nitrosodimethylamine. Lung tumors were also promoted by 2,2',3,4,4',5'-hexachlorobiphenyl, a prominent constituent of Aroclor 1254 and an agonist of the Ah receptor; this action was partially abrogated by cotreatment with 2,2',4,4',5,5'-HCB, an Ah receptor antagonist. To discover whether longterm induction of cytochromes P450 regulated by the Ah locus occurred after single-dose treatment, adult male mice were given Aroclor 1254 or TCDD at two different dose levels and sacrificed at time intervals from 48 hrs to 30 weeks. Induction of cytochromes P450 IA1 and IA2 isoforms, as indicated by specific enzyme assay and Western immunoblots, was persistent in both liver and lung for at least 12 weeks (TCDD, 50 nmol/kg) or 30 weeks (Aroclor, 500 mg/kg). With a lower TCDD dose (5 nmol/kg) induction was maintained longer in lung than in liver. These data confirm that single exposure to chlorinated aromatic hydrocarbon elicit sustained biochemical and tumorigenic responses, and suggest that the lung may be a sensitive target of this exposure.

INTRODUCTION

Polychlorinated biphenyls and dioxins cause tumors in laboratory animals after repeated or chronic exposure and also promote the development of tumors initiated by genotoxic carcinogens. The human health risk associated with the lipophilic, metabolism-resistant PCB and dioxin congeners, present at detectable levels in tissues of most humans, is of particular concern. As a model for study of tumorigenic and other toxic effects of these bioaccumulated congeners, this laboratory has studied tumor promotion and associated biochemical changes in mouse tissues after a single dose of PCBs or the dioxin, TCDD. In an earlier

study, a single dose of Aroclor 1254 (500 mg/kg) promoted the development of lung tumors and the growth of liver neoplasms initiated by N-nitrosodimethylamine (NDMA) (1). Recent efforts confirm this finding and indicate that longterm changes in cytochromes P450 occur in target organs after a single dose of PCBs or TCDD. Two isoforms of cytochrome P450 were followed by specific enzyme assay and Western immunoblotting: IA1, the form induced by polycyclic aromatic hydrocarbons, and IIB1, responsive to phenobarbital-like compounds. Both isoforms have been implicated as markers for or contributors to tumor promotion.

EXPERIMENTAL FINDINGS

Tumor Promotion. Numbers of lung and liver tumors were quantified in male Swiss mice after initiation with 5 mg/kg NDMA on postnatal day 4 followed by a single dose of Aroclor 1254 (250 mg/kg) on day 8. The PCBs treatment resulted in a significant 2-6-fold increase in numbers of liver and lung tumors at 7 and 12 months after treatment. At 18 months the difference was no longer seen. In a parallel study, amounts of 9 PCB congeners were quantified in liver, lung and carcass at intervals after treatment by capillary gas chromatgraphy with electron capture detection (Drs. S. Fox and H. Issaq). Notably, 2,3,3',4,4'-PCB was selectively retained in liver and lung compared with carcass, and 2,2',3,4,4',5'-HCB had relatively higher levels in lung. Both congeners are Ah-receptor agonists. In a study of tumor promotion by specific congeners, 2,2',4,4',5,5'-HCB an Ah-receptor antagonist, did not promote lung tumors, whereas 2,2',3,4,4',5'-HCB did promote, and its promotive action was abrogated by simultaneous exposure to 2,2',4,4',5,5'-HCB (2). These findings confirm that PCBs promote tumors after bioaccumulation and implicate Ah-receptor activation in this process.

Induction of Cytochromes P450 in Target Organs. Adult male Swiss mice were injected i.p. with Aroclor 1254 (100 or 500 mg/kg), TCDD (5 or 50 nmol/kg) or olive oil and sacrificed at time intervals ranging from 48 hrs to 30 wks. Induction of cytochrome P450 IA and IIB1 activities were measured fluorimetrically by the dealkylation of ethoxy- and benzyloxyresorufin, respectively. Confirmation of this enzymology was provided by Western immunoblotting of lung and liver microsomes using monoclonal antibodies specific for cytochromes P450 IA (1-7-1) and IIB1 (2-66-3) (in collaboration with Drs. S. Park and H. Gelboin). Liver weights were elevated significantly for 1 week and for 12 weeks after the low and high TCDD doses, respectively. Amount of total cytochrome P450 was significantly elevated in liver, especially at the

higher dose (2-fold at 1 week). Exposure to either dose of TCDD significantly elevated ethoxyresorufin-O-deethylase (EROD) activity (IA specific) in lung homogenates at every time point investigated, with activity still 10-fold greater than controls 12 weeks after the lower dose. Hepatic EROD was also persistently elevated at the high dose, but decreased to near basal values by 12 weeks after the lower dose. Densitometric scanning of Western blots from both tissues confirmed this enzymology. Benzyloxyresorufin-O-dealkylase (BenzROD) was also elevated about 2-fold in both tissues, although Western immunoblot analysis did not demonstrate any increase in IIB1 protein. In mice treated with low or high dose Aroclor 1254 and examined over a 30 week time course, EROD activity in lung was significantly elevated at all time points in both dose groups, reaching a maximum 1 weeks after treatment. After 500 mg/kg there was no significant decline in this induced level over 30 weeks. With the 100 mg/kg dose there was a sharp decrease by 12 weeks. Specific IIB1 induction was not observed in lung homogenates after either dose, and there was an actual 2-fold decrease in this enzyme activity at the early time points following high dose exposure. This represssion was confirmed by immunoblotting. Quantitative analysis of tissue PCB content revealed that two hexachlorobiphenyls predominated in lung homogenates after 30 weeks, namely 2,2',3,4,4',5'-HCB and 2,2',4,4',5,5'-HCB which together accounted for approximately 60% of total lung PCBs. In contrast to results obtained after Aroclor treatment of infant mice, none of the congeners were selectively retained in lung.

Discussion

These data demonstrate that a single dose of the PCB mixture Aroclor 1254, a prominent congener in the mixture, 2,2',3,4,4',5'-HCB, or TCDD has persistent cellular effects in rodent liver and lung related to tumor promotion. In the experiments with infant mice, several results seemed to point at involvement of the Ah receptor in the promotion: selective retention of Ah-receptor agonist congeners in liver and lung; promotion of lung tumors by one of these; and abrogation of promotion by a receptor antagonist congener. Since activation of the Ah receptor leads to induction of the family IA cytochromes P450, we have begun systematic examination of induction of this family after single-dose exposure to PCBs and to the prototype Ah receptor agonist, TCDD. The results thus far amply confirm that cytochromes P450 IA remain induced in liver and lung for many weeks after a single dose of TCDD or Aroclor. With the lower (5 nmol/kg) dose of TCDD, cytochrome P450 IA remained elevated over controls in lung

for at least 12 weeks, while activity in liver had returned to control levels by this time. Although several factors may contribute, it is likely that more complete and rapid clearance of the TCDD from liver explains this difference. Thus lung is expected to be an especially sensitive target for the toxic effects of residual TCDD.

The prolonged induction was even more striking after a single 500 mg/kg dose of Aroclor: a 25-fold induction of EROD and cytochrome P450 IA protein in lung had not significantly declined even after 30 weeks. The persistence of the effect was dose dependent, since a steady decline occurred after 4 weeks following the 100 mg/kg dose. Interestingly, IIB1 activity, which is constitutively expressed in rodent lung, was actually inhibited by high dose PCB exposure. The inhibition observed did not appear to be related to the level of IA induction in this tissue, suggesting that the presence of IA-inducing isomers was not entirely responsible for the inhibition of IIB1. The rebound of IIB1 activity by 12 wks may be related to the clearance of certain isomers responsible for this effect, although this has not been definitively demonstrated.

Selective retention of certain Ah receptor-agonist PCB congeners in liver and lung after treatment of infant mice with Aroclor 1254 was a notable finding and may reflect the presence of specific binding proteins in these organs. Current findings suggest that this selective accumulation does not occur after treatment of adult mice. Tumor promotive effects of the PCB congeners in adult mice are currently under test. Special sensitivity of newborn mice would be of particular interest in light of the ready delivery of PCBs in human breast milk.

The research in progress presented here confirms that chlorinated environmental chemicals stored in bodies contribute to tumor promotion and suggest that activation of the Ah receptor and increased cytochromes P450 IA are somehow involved, or at least markers for the persistent toxic effects. Continued analysis of this model should be helpful in elucidation of these mechanisms.

References

1. Anderson, L.M., Ward, J.M., Fox, S.D., Isaaq, H.J., and Riggs, C.W. 1986. Intl. J. Cancer 38, 109-116.
2. Anderson, L.M., Beebe, L.E., Fox, S.D., Issaq, H.J., and Kovatch, R.M. 1990. Promotion of mouse lung tumors by bioaccumulated polychlorinated aromatic hydrocarbons. Exp. Lung Res., in press.