Assessment and Implications of PCB Estrogenicity

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Introduction

The role of background levels of various industrial chemicals and other environmental pollutants in alteration of human and wildlife endocrine systems has recently expanded as a topic of public health concern. Published studies indicate or suggest that exposure to these "endocrine disruptors" result in a multitude of effects which reflect the pleopotent nature of normal endocrine functions. This has been the continued topic of reviews and comments[1,2] which discuss implications for cancer etiology, reproductive and gestational deficits, and developmental problems including sexual disfunction, neurotoxicity, and immunotoxicity due to chronic or acute exposure. Reports range from human and wildlife epidemiological studies to laboratory based animal and in vitro studies and include cancer development as well as non-cancer effects which have recently become recognized as critical to a complete public health evaluation.

Modulators of estrogen function have been the object of intense study within the arena of endocrine disruptors. The correlation between the effects of estrogen disfunction and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure in animals has been proposed as a general explanation of TCDD toxicity from both a species dependent and species independent point of view[3]. TCDD is a potent inhibitor of estrogen mediated activity which has been shown to be Ah receptor dependent as reviewed by Safe[4]. The precise mechanism of this activity is unknown but is considered to involve factors such as target organ intracellular E_2 depletion through induced P450 mediated metabolism, possible intervention at the level of xenobiotics and estrogen response elements, or, in the case of some animal studies, down regulation of the estrogen receptor in liver and uterine tissue which may be secondary to E_2 depletion through metabolism. The biological effects of PCBs are often similar to, although less potent than those of TCDD as reviewed by Safe[5]. An additional public health complication is based on the observation that certain PCBs or mixtures of PCBs exhibit no antiestrogenic activity but are actually estrogenic.

The focus of this laboratory has been on alteration of estrogenic activity in human breast tissue by TCDD[6-11]. The MCF-7 cell line, which is derived from a human adenocarcinoma of the breast, is well established as a model of estrogen-responsive cells. MCF-7 cultures respond to estrogen by increases in the expression of a number of genes. We have shown that MCF-7 cells used in this laboratory exhibit localized estrogen dependent postconfluent cell proliferation, which results in cellular aggregation or multilayered nodules of cells termed foci[6]. This foci development may be a response to the estrogen growth stimulus which characterized the original breast epithelial precursor of this tumor cell line. As such, estrogen focus development in MCF-7 cells may represent the basic characteristics of an estrogenic response, i.e. induction of concerted gene expression resulting in tissue restructuring through enhanced postconfluent cell proliferation. Since the foci are easily counted, their development is being investigated

as a relevant human tissue based assay for estrogenic potency. This report demonstrates the usefulness of the MCF-7 focus development as an assay for the evaluation of positive and negative estrogen modulation by a PCB and a hydroxylated PCB acting through Ah receptor dependent and independent mechanisms.

Methods

Estrogen assay : Estrogen induction of MCF-7 foci was undertaken as described previously[8]. Briefly, stock MCF cells were suspended in Delbeccos modified Eagles medium supplemented with 5% bovine calf serum (DC_5) by treatment with 0.25% trypsin in phosphate buffered saline (PBS). These cells were seeded into 24-well plastic tissue culture plates (2 cm^2/well) at a density of 10^5 cells per well in 1 ml of medium, incubated for 24 hr and refed then and every 4 days thereafter with 2 ml of DC_5 with the test compound concentration series and E_2, or DMSO. DC_5 has previously been shown not to support foci development[8]. After 14 days the cultures were fixed with formalin in PBS, stained with 1% Rhodamine B for foci enumeration using a New Brunswick automated colony counter modified to magnify the image of the multicellular foci. The foci retain the red Rhodamine B strain to a greater extent than do the surrounding monolayer cells, affording an appropriate contrast for counting.

Antiestrogen Assay: It was anticipated that certain PCBs (co-planar) will be antiestrogenic and it is possible that some hydroxylated PCBs may also exhibit antiestrogenic activity. These possibilities were examined by the efficacy of these compounds to inhibit focus development elicited by 10^{-10}M E_2 (the approximate EC_{50}) for focus development.

Results

Estrogen Induction of MCF-7 Foci: An estrogen assay was undertaken using a dilution series of 17β-estradiol from 10^{-8} to 10^{-12}M as a positive estrogen calibration control. The results shown in figure 1 indicate maximal stimulation of foci with 1 nM E_2 and an EC_{50} of between 0.1 and 0.01 nM These results are consistent with those reported previously[8]).

TCDD Suppression of 17β-Estradiol Induced MCF-7 Foci: An antiestrogen assay was performed using a dilution series of TCDD from 10^{-8} to 10^{-10} M in 0.1 nM E_2 as a positive antiestrogen calibration control. The results shown in figure 2 (filled circles) are consistent with the TCDD suppression of E_2 induced foci previously reported[9] indicating consistent assay sensitivity.

Antiestrogenic Activity of 3,4,5,3',4' Pentachlorobiphenyl (PeCB): Various concentrations of 345,3'4' PeCB were tested for antiestrogenicity in parallel with TCDD. The results shown in figure 2 (open circles) indicate that this PCB congener is antiestrogenic in the MCF-7 focus assay with an IC_{50} of about 1μM.

Estrogenicity of 4 0H-2',4',6' Trichlorobiphenyl (TCB): The estrogenicity of a five-fold concentration series of 4 0H-2',4',6' TCB was tested. The results, shown in figure 3, indicate that this PCB metabolite exhibits estrogen activity with an EC_{50} of about 0.1 μM.

Discussion

The preliminary studies reported here demonstrate that the coplanar 3,4,5,3',4' PeCB exhibits

antiestrogenic activity with a potency of about 0.01% of that of TCDD. This antiestrogenicity is consistent with the Ah receptor mediated dioxinlike activity associated with this congener. The results also demonstrate the estrogenic activity of 4 0H-2',4',6' TCB, indicating a potency of about 0.01% of that of E_2. This activity, as discussed below, is probably mediated through an Ah receptor independent mechanism.

The ability of environmentally-derived xenobiotics to express estrogenic activity in mammalian systems was established over 20 years ago. PCB commercial mixtures, particularly those with lower percentage chlorine content, exhibited estrogenic responses as assessed by the 18-hr glycogen response of the immature rat uterus[12]. Subsequently, administration of PCB mixtures was shown to extend the oestrus cycle, decrease the frequency of implanted ova in mice[13], and produce significant uterine growth in weanling rats[14]. Initial attempts to determine which PCB congeners in the PCB mixtures were responsible for estrogenic activity involved acute administration of the PCBs individually to immature rats[15]. Estrogenicity was assessed by measuring uterine weight, percent water and percent glycogen in the immature uterus. Only 2-chlorobiphenyl produced significant changes in all three parameters, and 2,2'-dichloro- and 2,4,6,2',4',6'-hexachlorobiphenyl caused increases in tissue weight only. Biphenyl, 3- and 4-chlorobiphenyl, 2,4'-, 3,3'- and 4',4'-dichlorobiphenyl, 2,4,2',4'-, 2,5,2',5'-tetrachlorobiphenyl and 2,4,5,2',4',5'-hexa-chlorobiphenyl yielded inconclusive results. Overall the estrogenicity of the PCB congeners investigated did not account for the extent of the estrogenicity of the commercial mixtures of PCBs.

A mechanism of this estrogenic activity was proposed after an assessment of the estrogenicity of PCB congeners was conducted by Korach et al.[16]. These authors assumed that PCBs expressed estrogenicity only after metabolism to 4-hydroxylated metabolites, based on model studies comparing the structures of 4-hydroxy PCBs with estrogen. Studies comprised determinations of the binding activities of hydroxylated PCB congeners to the estrogen receptor. Actual determinations of estrogenic effects in mice were made only with 4-hydroxy-2',4',6'-trichlorobiphenyl and 4,4'-dihydroxy-3,5,3',5'-tetrachlorobiphenyl, which indicated that the former compound is estrogenic while the latter is not. Structure-estrogenicity relationships of the PCB hydroxylated metabolites - based on estrogen receptor binding affinities suggest that, in the case of para hydroxylation, ortho-chlorine substitution was critical to enhanced binding of the estrogen receptor with other chlorine substitution causing modulation of potency but specific relationships were not developed.

While restricted rotation around the phenyl-pheynl bond, brought about by ortho-chlorine substituents, and para hydroxy substituents on PCBs both enhance binding to the estrogen receptor, many other aspects of structure-estrogenic activity remain to be evaluated. A recent study was performed by Jansen et al.[17], which evaluated the effects of various PCB congeners and PCB mixtures on immature rodent rat uterus in vivo, which is known to increase in wet weight and proliferative activity as a response to 17β-estradiol. The nonplanar PCB (2,5,2',5'-TCB, the PCB mixture Aroclor 1242, and the hydroxylated 4'OH-2,4,6,-TCB caused increases in these parameters while the coplanar 3,4,3',4'-TCB suppressed estrogenic activity of 17β-estradiol and Aroclor 1242. Further development of PCB structure-activity relationships will be essential for predicting which of the PCBs present in the environment could potentially function as estrogens in humans. Thus assessment of PCB estrogenic activity in human tissue, the structure-activity relationships involved, and the role of metabolism are all issues which require investigation. The results of the studies reported here demonstrate the potential usefulness of the human breast cell MCF-7 focus assay to aid in this assessment.

Supported by NIEHS Superfund Project 2P42ESO4913.

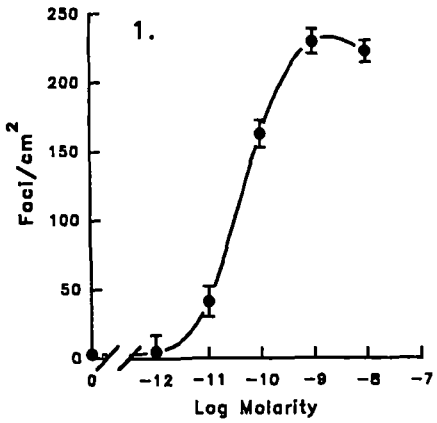

Figure 1. Dose response induction of MCF-7 foci development by 17β-estradiol (E_2). MCF-7 culture were treated with the indicated concentrations of E_2 over 14 days and foci were enumerated as described. Mean of four replicates of a representationexperienced ± standard deviation.

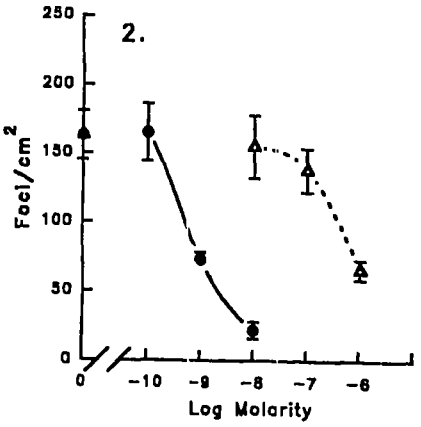

Figure 2. Suppression of 0.1 nM E_2 induction of MCF-7 foci by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or 3,4,5,3',4', pentachlorbiphenyl (PeCB). MCF-7 cultures grown in the presence of 0.1 nM E_2 were treated with the indicated concentrations of TCDD or PeCB over 14 days and foci were enumerated as described. Mean of four replicates of a representative experiment ± standard deviation.

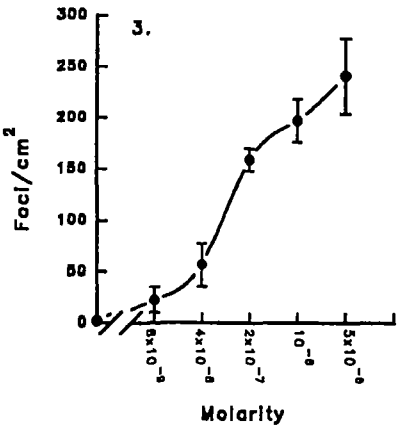

Figure 3. Induction of MCF-7 foci by 40H-2',4',6'-trichlorobiphenyl (TCB). MCF-7 cultures were treated with the indicated concentration of TCB over 14 days and foci were enumerated as described. Mean of four replicates of a representative experiment ± standard deviation.

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