Antiestrogenic activity of 3,3'4,4'-tetrachlorobiphenyl and 3,3'4,4'5-pentachlorobiphenyl in T47D breast cancer cells

Mona S. Gupta, Kavita Ramamoorthy and Stephen Safe

Department of Veterinary Physiology and Pharmacology, Texas A&M University
College Station, Texas 77843, USA

Introduction

Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) are industrial compounds or by-products which have been identified as contaminants in almost every component of the global ecosystem [1]. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is the most toxic member of this class of halogenated aromatic hydrocarbons and has been used as a prototype to investigate the toxic and biochemical effects produced by this class of chemicals [2]. Initial studies showed that TCDD binds with high affinity to the aryl hydrocarbon receptor (AhR) protein. Subsequent studies have identified a large number of structurally-related compounds including PCB, PCDD and PCDF congeners which also bind to the AhR. TCDD and related compounds elicit a broad spectrum of biochemical and toxic responses in the laboratory animals and cultured mammalian cells. AhR agonists have been extensively characterized as endocrine disrupters.

Nesaretnam and coworkers [5] recently reported the estrogenic activity of 3,3',4,4'-tetrachlorobiphenyl (tetraCB) in human breast cancer cells and in the immature mouse. However, previous studies have shown that tetraCB inhibited E2-induced secretion of procathepsin D in MCF-7 human breast cancer cells [3]. Recent studies in our laboratory also suggest that neither tetraCB nor 3,3',4,4',5-pentachlorobiphenyl (pentaCB) was estrogenic in the mouse uterine model and in contrast, exhibited some antiestrogenic activity. This study further investigates the estrogenic activity of tetraCB and pentaCB, both well-characterized AhR agonists, in T47D human breast cancer cells.

Materials and Methods

Cell Proliferation Assay: T47D cells were obtained from the American Type Culture Collection and maintained in an α minimum essential medium (α MEM) supplemented with 1.2 g/L bicarbonate, 5% FBS, pH 7.4, 10ml/L antibiotic solution. Cells were seeded in 6-well plates (50,000/well) in DME-F12 supplemented with 5% FBS treated with dextran-coated charcoal, 1.2g/l $NaHCO_3$ and 10ml/L antibiotic solution. Cell proliferation was determined as previously described [4].

Transient Transfection Assay

The following plasmids which contained the bacterial chloramphenicol acetyl transferase (CAT) reporter gene were used in this study: pCKB-CAT which contained a 2.9 kb fragment from the rat creatine kinase-B gene promoter ; pCATD-CAT which contained 2.74 kb human cathepsin-D gene 5'-promoter, cloned into PBLCAT2. The human ER

(hER) expression plasmid was also transfected with the E2-responsive constructs. Transient transfection assay was performed according to procedures previouly described [6].

Competitive ER Binding Assay

Mouse uterine cytosol was incubated with 20nM [^{3}H] E2 in the presence or absence of varying concentrations of unlabeled E2, tetraCB or pentaCB and competitive ER binding assay was determined as previously described [4].

Results and Discussion

1. The results in Figure 1 show that tetraCB alone (1-1000nM) did not induce proliferation of T47D cell whereas 1nM of E2 caused a 3-fold increase in cell growth. In cells cotreated with 1nM E2 plus 1-1000nM tetraCB significant inhibition of hormone-induced proliferation was observed at the lowest concentration of tetraCB (1nM). Similar results were also observed for the coplanar pentaCB congener (Figure 2).

2. In T47D cells transiently transfected with pCD 1nM E2 induced CAT activity (8-fold) whereas 1000nM tetraCB or pentaCB alone had no effect on CAT activity (Figure 3). In cells cotreated with E2 plus the antiestrogen ICI 182,780 there was complete inhibition of E2-induced CAT activity. In contrast, neither 1000nM tetraCB nor 1000 nM pentaCB exhibited antiestrogenic activity using pCD. These results are consistent with previous studies showing that TCDD does not inhibit E2-induced cathepsin D secretion in T47D cells (P. Fernandez, unpublished results).

3. In contrast, both 1000nM tetraCB and 1000nM pentaCB and ICI 182, 780 inhibited induction of CAT activity in T47D cells, treated with 1nM E2, transiently transfected with pCKB (Figure 4).

4. The failure of tetraCB and pentaCB to induce estrogenic responses in T47D cells is also consistent with their failure to bind the ER (Figure 5) and contrasts with results previously reported by Nesaretnam [5]. The rationale for differences between results reported in this paper and previous studies is unknown but is currently being investigated in this laboratory.

References

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Acknowledgements

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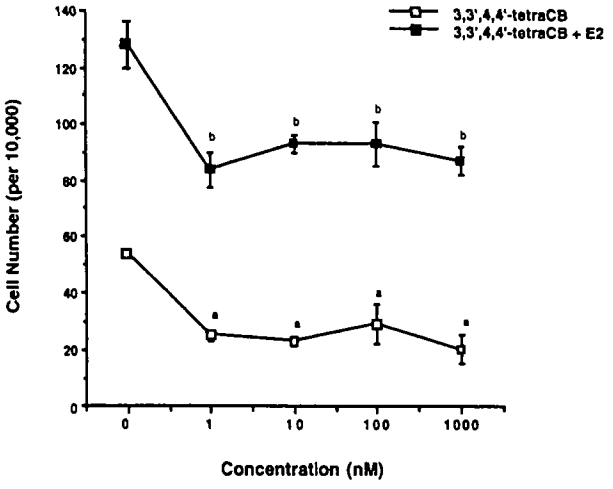

Figure 1 Cell Proliferation Assay: Estrogenic and antiestrogenic activity of 3,3',4,4' tetraCB in T47D cells

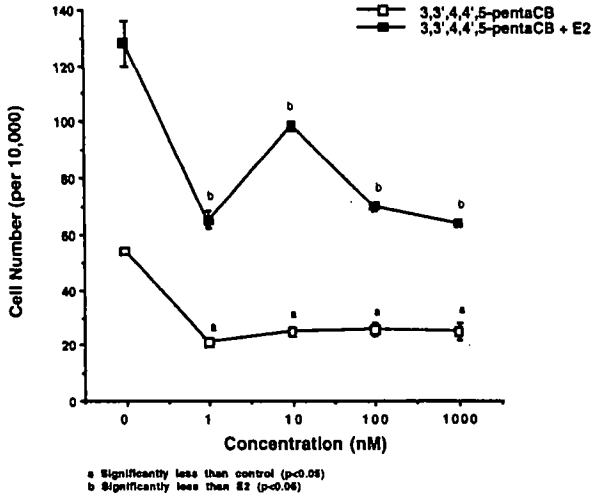

Figure 2 Cell Proliferation Assay: Estrogenic and antiestrogenic activity of 3,3',4,4',5 pentaCB in T47D cells

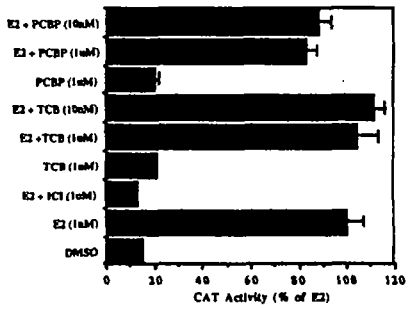

Figure 3. Antiestrogenic activity of 3,3',4,4' tetraCB and 3,3',4,4',5 pentaCB in T47D cells transiently transfected with pCD2.74

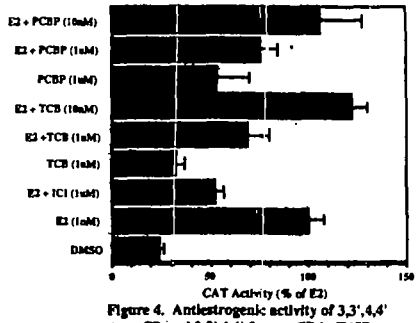

Figure 4. Antiestrogenic activity of 3,3',4,4' tetraCB and 3,3',4,4',5 pentaCB in T47D cells transiently transfected with pCKB

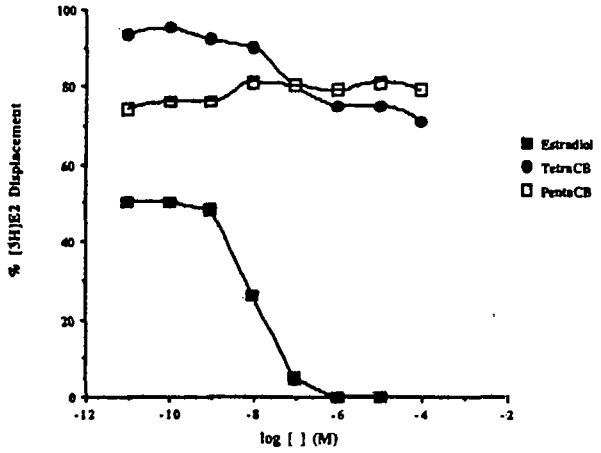

Figure 5. Lack of competitive binding of tetraCB and pentaCB to mouse uterine cytosolic ER.