APPRAISAL OF 18 CHRONIC, MULTIDOSE, MULTIRESPONSE BIOASSAYS OF PCBS OR DIOXIN IN S-D RATS INDICATES SINGLE MODE-OF-ACTION FOR TUMORIGENESIS

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Introduction

Reports of two chronic multidose bioassays of TCDD in male or female Spartan Sprague-Dawley (S-D) rats;[1] of eight such bioassays of Aroclors 1016, 1242, 1254, or 1260 in male or female Charles River S-D rats;[2] and of eight such bioassays of TCDD, PeCDF or PCBs 126, 153, or 118 or combination thereof in female Harlan S-D's[3] have included data on tissue PCB and/or dioxin equivalent (PCB/TEQ) accumulation and concomitant biochemical changes. A previous investigation of inter-response relationships for the Aroclor-dosed rats showed the total hepatotumorigenesis (HT; mostly hepatocellular adenomas) to be closely, consistently, and predictively correlated, in both the dosed and undosed animals, with the net hepatic cytosolic activity of redox-cycling quinones (RCQ) as catalysts for the production of reactive oxygen species (ROS; initially, $O_2^{\bullet-}$, then H_2O_2). This indicated a tumorigenic mode of action (MOA) whereby tissue PCB/TEQ accumulations induced (or inhibited) expression of mixed function oxidases (MFO; e.g., P450 cytochromes), which can convert endogenous precursors to RCQs, resulting in ROS-mediated promotion (proliferation) of spontaneously initiated (mutant) liver cells.[4] In order to further characterize the nature and scope of this MOA we have now extended the inter-response correlation studies to all the other reported chronic bioassays of PCB/TEQ in S-D rats.

Methods

Available indicators of MFO induction (CYPs 1A1, 2B1/2; EROD, PROD, A4H; changes in PCB congener levels or ratios) and of tissue PCB/TEQ activities (mean midlife or 53-week lipid- or fatty tissue-normalized ΣPCB or TEQ) for each sex were plotted against each other to indicate approximate EC50 and IC50 values for MFOs induced or inhibited. Cumulative incidences of individual or grouped tumor types were plotted against such tissue PCB/TEQ or MFO indicators, or combinations thereof, to indicate forms of the tumor incidence vs. PCB/TEQ dependencies and identities of specific MFOs involved for the various dose−S-D subtype−sex combinations.

Results and Discussion

Interstrain differences and simularities. – At high doses, tumorigenesis was increased at 4-6 sites and decreased at 6-9 sites, with little net effect on total tumor incidence. The plots of tumor incidence vs. lipid ΣPCB or TEQ showed a dozen different patterns, none of them the classic linear-through-zero, positive slope, no threshold pattern expected for tumorigenic processes mediated by mutagenesis or by activation of a single receptor. The plots differed by S-D rat substrain, as well as by sex and dosing agent. The Harlan females exhibited less tumorigenesis at low tissue TEQ than the others, but more at higher TEQ (plots curved upwards rather than downwards). This appeared correlated with lack of low EC50, ΣPCB-induced expressions of MFOs carrying out PCB and PROD metabolism in the Harlan rats, but increased non-saturating induction of MFOs with high EC50's. Plots of EROD induction and of hepatic tumor incidence vs. log TEQ were both sigmoidal, but with the EC50's for tumorigenesis 10-20 times those for EROD induction, indicating requirements for additional PCB/TEQ-induced activities. In rats dosed with PCB 126-153 mixtures, the curves moved closer together, indicating that the non-dioxin-like PCB 153, which was non-tumorigenic by itself, could complement the CYP1A1/EROD activity induced by PCB 126. Modeling showed the liver and gingival tumors in the Harlan females dosed with PCB 126-153 mixtures to occur in direct proportion to the product [EROD][PCB153]; in those dosed with PCB 126 alone, to the product [EROD][PCB 126]; and for the cystic keratinizing epithelioma (CKE) tumors of the lung, where EROD expression was minimal, to $[\text{PCB 126}]^2$. Analysis of the data for Aroclor-dosed Charles River females [4] showed the limited tumorigenic response to Aroclor 1016 to be proportional to $[\Sigma\text{PCB}]^2$, and those to Aroclors 1242 and 1254 to show displacements between the EC50s for CYP1A1 induction and tumorigenesis, indicating again that more than one PCB/TEQ response was required for tumorigenesis in S-D females.

Further Characterization of MOA. – The observations of high EC50 (non-saturating) inductions of MFOs with TCDD-, PeCDF-, or PCB 126-metabolizing activities add two more to the previous list [4] of six MFOs inducible in either male or female S-D rats, by either ΣPCB or TEQ, usually with at least partial inhibition by the other. The mass action dependencies indicated that in some cases a single MFO (or a covariant pair) could induce RCQ activity and tumorigenesis, whereas in others two were required. It may be noted that the conversion of estrogen to an RCQ (e.g., a glutathionylated estrogen quinone) requires two types of oxidative activity: (1) aromatic hydroxylase activity, also indicated by PCB metabolism, (designated MFO_o), and (2) catechol-to-quinone oxidation, which requires only electron abstraction (designated MFO_e), We concluded that the requirement for two MFOs in the S-D females, but only one in the males [4], results from the utilization of the phenol, estrogen, as the RCQ source in the females, but a different endogenous substrate in the males. The contribution of RCQ activity to ROS production in the Harlan females was not measured, but the total ROS formation was found correlated with tumorigenesis, [5] as had been found for RCQ-derived ROS (most of the total ROS formation [4]) in the Charles River females.[4] Thus, an MOA of the MFO-RCQ-ROS generic type appeared involved in all types of PCB/TEQ-increased tumorigenesis in S-D rats, despite the variations in tumor incidence vs. tissue PCB/TEQ patterns.

The suppressions of prostate, pituitary, and other tumors in the males, and of mammary tumors in the females (which must be explainable in any proposed MOA) were clearly correlated with CYP1A1/EROD induction, but whether mediated by MFO inhibitions or simply the known downregulation of the estrogen and androgen receptors[6] was not determined. The suppressions of other types of extrahepatic tumors in the Charles River and Harlan females were mediated by processes not correlated with EROD induction. The inhibitions of increased hepatumorigenesis at sub-threshold Aroclor doses in Charles River males and females were previously linked to suppression of constitutive MFOs. [4] This process was more strongly evident in the TCDD-dosed Spartan males and females, which showed clear hormesis at low doses. [1]

Further mediators and substrates for tumor promotion. – The present findings provide additional evidence that in rats PCBs and dioxins can promote the development of tumors derived from certain types of initiated cells, and that the first five steps of this process lead from tissue PCB/TEQ accumulation to ROS production. Correlation of ROS production with the mitotic stimulation (promotion) of initiated cells has been long recognized, but not the nature of the initiated cells or the actual steps leading from ROS production to tumor promotion and progression. Recent findings by others suggest biologically plausible answers to both questions.

First, over the past two decades there has been an enormous accumulation of information about the signaling pathways by which activation of receptor tyrosine kinases (RTKs) leads to mitosis. [7,8] RTK activation was initially found to be mediated by binding to bivalent ligands (e.g., polypeptide mitogens, or growth factors) resulting in dimerization and autophosphorylation of the RTK. [7,8] More recently, it has found, particularly for membrane-spanning RTKs that carry extracellular cysteine-rich domains, [8] such as EGFR [9] and IGFR [10], that the dimerization/activation can be effected not only by ligands such as EGF, TGF-α, IGF-1, and IGR-2, but also by the ROS, H_2O_2, [9,10] presumably acting on the cysteines. This means that any system that generates ROS, such as the PCB/TEQ-induced MFO-RCQ-ROS system in S-D rat liver, or the benzo(a)pyrene quinone (an RCQ)-induced pathway in breast cancer cells [12] can stimulate the multi-step pathway [7,8,12] leading from RTK activation to mitosis. In addition, RTKs have also been found to be activated by steroid-bound sex hormone receptors, [11] but these to be downregulated by dioxin-like inducers of CYP1A1/EROD. [6] Thus, the PCB/TEQ-suppressible development of mammary and prostate tumors, as well as the increased S-D rat liver tumors, may all proceed via conventional RTK-mediated mitotic signaling processes.

As for the nature of the PCB-promotable initiated cell, the earliest genetic alteration seen (in GGT^+ hepatic foci from PCB 52 + PCB 77-dosed S-D rats) was a duplication at chromosomal position 1q41, [13] which includes the gene for 1GF-2, and indeed, increased expression of 1GF-2 was observed. [13] More recently, it has been found that increased expression of 1GF-2 rather counterintuitively increases expression of its receptor, 1GFR, [14] an RTK that is also one of those that can be activated by ROS. [10] Thus, this mutation would likely render the cell more responsive than a normal cell to inducers of ROS, such as PCB/TEQ.

Chart 1 summarizes the characteristics of most of the steps leading from PCB/TEQ accumulation to S-D rat liver tumorigenesis, not including those in the multiple pathways running between even one RTK (e.g., EGFR) and mitosis. [7] Also not included are the numerous homeostatic processes that can result in hormesis, thresholds, and tumor suppression.

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Chart 1. Characteristic Features of PCB/TEQ-Modulated Tumorigenesis in S-D Rats

1. *Event sequence and correlates*

PCB/TEQ*→NR→MFO*→RCQ→*ROS→RTK→mitosis*→tumors

*Tissue level or activity quantitatively correlated with tumorigenesis

2. *PCB/TEQ accumulation in tissue*

With chronic dosing, PCBs, TEQs rise to mid-life steady state levels that correlate with responses; effects on tumorigenesis vary with sex, strain, site, and PCB/TEQ type.

3. *Nuclear receptor (NR) activity modulation*

MFOs and microarray covariance groups indicate PCB/TEQs modulate multiple NR transcription factors; activated NRs downregulate each other, resulting in hormesis, thresholds, and tumor suppression.

4. *Mixed function oxidase (MFO) expression*

Multiple MFOs (CYPs, etc.) induced or suppressed; two key MFO activities, MFO_o and MFO_e inferred; both needed for tumor promotion in S-D females, only MFO_e in males.

5. *Redox-cycling quinone (RCQ) production*

In females, glutathionylated estrogen quinones formed via action of MFO_o and MFO_e on estradiol and GSH; in males RCQs formed via MFO_e activity on other endogenous species.

6. *Reactive oxygen species (ROS) production via RCQ*

RCQs catalyse $O_2^{\bullet-}$ production by 1-electron shuttling between NADPH-reduced microsomal flavoprotein and O_2; $O_2^{\bullet-}$ converted to H_2O_2 by SOD; minor H_2O_2 conversion to $HO^{\bullet}$.

7. *Receptor tyrosine kinase (RTK) activation by H_2O_2*

Cysteines in EGFR, IGFR, etc. oxidized by H_2O_2; [9,10] dimerization leads to autophosphorylation, binding of signaling molecules.

8. *Mitotic stimulation*

RTK activation leads to mitotic stimulation by several much-studied pathways [7,8]; also to inhibition of gap junction intracellular communication (GJIC). Both of which favor tumor growth.

9. *Tumor promotion*

Liver (and other) tissues contain appreciable numbers of spontaneously initiated cells that will not survive and proliferate unless promoted. PCB/TEQ increases mitosis (proliferation) of both normal and initiated (mutant) cells, but in the latter more so. The PCB/TEQ-promoted tumor cells show gene doubling at chromosomal position 1q41 and increased expression of 1GF-2[13], now known to upregulate the ROS-responsive RTK, IGFR[14], thus explaining the increased sensitivity of this type of initiated cell to ROS-producing tumor promoters.

10. *Tumor progression*

Charles River S-D females showed increased incidence of hepatocellular carcinoma in the groups with highest productions of ROS and depletions of the H_2O_2 scavenger, GPx. [4] This tumor progression was presumably mediated by DNA damage induced by $HO^{\bullet}$ formed by uncontrolled levels of H_2O_2.