The Biochemistry of Tumor Promotion and Inhibition in PCB-dosed Rats

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Introduction

Our group is currently near the end of a sizable 10-year effort to define the characteristics and mechanism of the production of tumors in rodents by chronic, maximum tolerated dose (MTD) administration of PCBs; and, by extension, MTD carcinogenesis by epigenetic lipophilic agents generally. This effort has included parallel multidose near-MTD lifetime (2-yr.) bioassays of four PCB compositions (Aroclors 1016, 1242, 1254, and 1260) in male and female Sprague-Dawley (S-D) rats for chronic toxicity and tumorigenicity (1, 2); determinations of PCB accumulations and congener distributions in various tissues (1, 3); measurements of a variety of enzyme activities and metabolite levels in liver specimens preserved after interim and final sacrifices (4,5); and interparameter correlations to define mechanistic sequences (6). By the time of last year's reports (5, 6) these studies had shown that PCBs had both tumorigenic and anti-tumorigenic activities (2); that both coplanar and non-coplanar PCB congeners could contribute to tumorigenicity, albeit via different mechanisms (1, 6); that both these mechanisms yielded quinonoid species that could redox-cycle in the cytosol to produce superoxide (4, 6); and that hepatic cytosolic superoxide production was both an early predictor and highly significant correlate of hepatotumorigenicity (4, 6). Since then we have learned: from histochemical studies (by J. Whysner, American Health Foundation, Valhalla, NY) that tumor promotion is correlated with a general increase in mitotic activity that becomes significant within the first year of PCB dosing; from measurements of various hepatic antioxidant levels and Phase II enzyme activities that the primary inhibitor of superoxide-mediated mitotic activity is glutathione; and from reports of other investigators of receptors that mediate mitotic stimulation by superoxide or derived reactive oxygen species (ROS) (7), and of lipid oxidation products that up-regulate expression of glutamate cysteine ligase (GLCL) and thence cytosolic glutathione (8). These old and new findings inspired a search for a coherent set of cause/effect relationships that could explain the development or inhibition of epigenetic carcinogenesis.

Results
The following deductions appeared consistent with all available data:

1. *The MTD tumorigenesis that is induced by PCBs or other persistent, bioaccumulative lipophiles is promotion-driven*. In classic two-stage tests, all such agents behave as tumor promoters rather than tumor initiators (9). Their role as growth regulators rather than mutagens is further supported by the observations of inhibitory as well as promotional responses (2).

2. *Tumor promotion results from increased mitotic stimulation*. This is evidenced by increased expression of proliferating cell nuclear antigen (PCNA) throughout the liver, bile duct hyperplasia, and appearance of $GSTP^+$ preneoplastic foci, all beginning between 6 and 12 months after the commencement of PCB dosing (before any increase in hepatocellular apoptosis) and correlated with late life hepatotumorigenesis.

3. *Tumor-promoting mitotic stimulation is signaled by cytosolic superoxide or a derived ROS*. We found that cytosolic superoxide was produced by mechanisms that were different for the PCB-dosed females, the PCB-dosed males, and possibly also the controls (1, 6) but in all cases there was a close and predictive correlation between superoxide production and hepatotumorigenesis (4). The mitogenic signal may be mediated by NF-κB or AP-1 (7).

4. *Cytosolic superoxide is produced by quinone-mediated redox cycling*. The redox-cycling agents responsible for increased superoxide production in the hepatic cytosols of S-D females have been identified as mixtures of glutathionylated estrogen quinones. Those doing likewise in the PCB-dosed males or male controls are also soluble, low molecular weight, and presumably quinonoid, but still unidentified.

5. *Quinone production results from oxidations by microsomal oxidases or oxidase-derived ROS*. The oxidations of estrogen to the 2,3- and 3,4-catechols, and thence to quinones are known to be mediated by oxidases of cytochrome P450 family 1. The ROS species also produced by cytochrome P450s of various families are known to attack phenols generally, producing both catechols and quinones.

6. *Microsomal oxidase production is induced by accumulation of PCBs and other lipophiles*. This is a very general and ancient response of multi-cellular animals to any accumulations of dietary lipophiles that are in the 50-500D molecular weight range, and not otherwise metabolizable (10). PCBs induce at least two groups of such oxidases (1, 4). The coplanar PCBs, acting through the Ah-receptor, induce the P450 family 1 oxidases that contribute to estrogen quinone formation and consequent tumorigenicity in S-D females. The non-coplanar PCBs, acting through a receptor for the phenobarbital-responsive enhancer module (PBREM), may induce the P450 family 2 and 3 oxidases, and perhaps NADPH oxidases as well, in both sexes.

7. *Microsomal oxidase/ROS causes lipid autoxidation as well as quinone production*. The autoxidation of microsomal lipids, especially those derived from polyunsaturated fatty acids, has been long known to be a free radical chain reaction that can be initiated by a variety of free radicals, including the ROS species formed by microsomal oxidases. The autoxidation products

include lipid hydroperoxides and derived molecular fragments such as malondialdehyde (measured as TBARS) and 4-hydroxy-2-nonenal (4-HNE).

8. *Lipid autoxidation products signal the up-regulation of GLCL and glutathione*. The induction of GLCL, the enzyme that controls glutathione formation, has been recently found to be mediated by 4-HNE, acting on an unidentified receptor for an ARE/EpRE response element (8).

9. *Glutathione can limit cytosolic ROS production and thence tumor promotion*. Glutathione is known to react readily with superoxide (k_2, $7.7x10^5$ L/mol sec) (11) as well as other ROS. We observed that superoxide production by hepatic cytosols under redox-cycling conditions could be reduced by added glutathione. More importantly, we found 50-200% increases in glutathione in the livers of all PCB dose groups that showed either the same or less hepatotumorigenesis than the controls. Such increases have also been seen in response to many other types of carcinogens (8). In the past, a possible role for glutathione in inhibiting tumor initiation has also been proposed, since glutathione is a trap for the electrophiles that alkylate DNA as well as for ROS.

Discussion

For the past three decades the biochemical basis for the induction of cancer by highly mutagenic chemicals has been known to involve the production of reactive electrophiles that could alkylate DNA, resulting in somatic mutations and tumor initiation. However, the bases for the more commonly observed production of animal tumors by non-mutagenic lipophilic chemicals at the MTD, or of tumor inhibition at low doses (12), have remained obscure. The nine cause/effect relationships listed above now show that the primary response to chronic PCB accumulation in an animal is the induction of microsomal oxidases and their associated ROS. This oxidase/ROS induction then leads to at least three series of secondary biochemical events. One consists of the increased metabolism of susceptible PCB congeners, thus reducing tissue accumulations. A second is the oxidation and glutathionylation of endogenous phenolics, such as estrogen, to produce soluble quinones that can redox-cycle to form cytosolic superoxide, which supplies the mitotic signal for tumor promotion. A third consists of the ROS-initiated autoxidation of microsomal lipids to produce TBARS and 4-HNE, which signal the up-regulation of GLCL and thence glutathione, which limits the production of cytosolic superoxide and electrophiles. In the rodent liver, with its prodigious capacity for oxidase induction, this glutathione-mediated inhibitory response can be overwhelmed at high PCB accumulations, resulting in hepatotumorigenesis. At lower doses, however, the net hepatotumorigenic response could be inhibitory, and in extra-hepatic tissues inhibitory even at the MTD (2). Thus, the glutathione-mediated protective response would appear adequate for preventing epigenetic tumorigenesis except near the MTD.

References

1. Silkworth, JB, Mayes, BA, Fish, KM and Brown, JF, Jr.; *Organohalogen Compounds*, **1997,** 34, 164.
2. Mayes, BA, McConnell, EE, et. al., *Toxicol. Sci*. **1998**, 48, 92.
3. Brown, JF, Jr., *Environ. Sci. Technol*. **1994,** 28, 2295.
4. Fish, KM, Silkworth, JB and Brown, JF, Jr., *Organohalogen Compounds*, **1997,** 34, 296.
5. Fish, KM, Silkworth, JB, and Brown, JF, Jr., *Organohalogen Compounds*, **1998,** 37, 113.
6. Brown, JF, Jr., Fish, KM, Silkworth, JB and Mayes, BA, *Organohalogen Compounds*, **1998,** 37, 67.
7. Sen, CK, *Biochem. Pharmacol*., **1998,** 55, 1747.
8. Liu, R-M, Gao, L, Choi, J, and Forman, HJ, *Am. J. Physiol*. **1998,** 275, 861
9. Shirai, T, *Toxicol. Path*. **1997,** 25, 453.
10. Nebert, DW, *Biochem. Pharmacol*. **1994,** 47, 25.
11. Wefers, H, and Sies, H, *Eur. J. Biochem*. **1983,** 137, 29.
12. Davies, JM and Svensgaard, DJ, in *Biological Effects of Low Level Exposures*, Calabrese, EJ, ed., Lewis Publishers, Boca Raton, **1994,** 67.