Enzymatic Bases for Tumorigenic Oxidant Production in the Livers of Rats Chronically Dosed with PCBs

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Introduction

The environmentally persistent organohalogen compounds, including the formerly commercial polychlorinated biphenyls (PCBs), are non-genotoxic, yet nevertheless produce tumors in laboratory rodents when chronically bioassayed at the maximum tolerated dose (MTD) (1).

In order to elucidate the mechanistic basis for this phenomenon, we commissioned parallel multidose MTD bioassays for chronic toxicity and tumorigenicity of Aroclors 1016, 1242, 1254, and 1260 in male and female Sprague-Dawley (S-D) rats (2), and correlated the observed hepatotumorigenicity with the levels and congeneric compositions of the tissue PCBs (3). Since then, we have been characterizing biochemical parameters in preserved livers taken at various time points during the bioassays and correlating them with liver PCB accumulations and tumor incidence. Last year, we discovered that the tumor incidence was closely correlated with production of cytosolic superoxide anion ($\cdot O_2^-$), a process mediated by as yet unidentified low molecular weight redox cycling catalysts (4). Here, we report the results of measurements, all by standard procedures, of various hepatic enzymes that were considered as possible agents for the production and control of this form of oxidative stress.

Results

CYP1 Isozymes. Mean levels of hepatic microsomal CYP1A1 protein and EROD and MROD activities (reflective of CYP1A1, CYP1B1 and CYP1A2, in various proportions) were all about twice as high in males as in females, but showed the same patterns of variation with Aroclor type and length of dosing (time) in both sexes. The inductions were high, dose-saturated, and TEQ-independent at 6 months, but subsequently declined and exhibited some TEQ and dose-dependence, especially in the 1016 and 1260 dose groups. These findings suggested dose-saturation of the DRE-mediated CYP1 isozyme transcriptions at 6 mo., subsequently partially relieved by down-regulation or inactivation of AhR.

CYP2B Isozymes. At all time points, inductions of CYP2B1/2 proteins and PROD and BROD activities were similar for all Aroclors and showed significant dose-dependency only for the Aroclor 1254-dosed females. At 6 mo., the inductions of CYP2B1/2 and PROD were similar for the two sexes, but subsequently dropped somewhat, especially in the females. Conversely, the inductions of BROD were greater in the males and remained constant with time in both sexes.

The general lack of Aroclor-dependence of these CYP2B metrics precluded the possibility of correlating them with tumorigenicity or cytosolic ROS production.

Microsomal ROS Production. Microsomal production rates of two reactive oxygen species (ROS), namely $\cdot O_2^-$ and peroxide (H_2O_2) were measured separately. At 6 mo. individual animals' productions of $\cdot O_2^-$ correlated closely with their CYP1A1 levels, but later $\cdot O_2^-$ increased relative to CYP1A1 in some animals with concommittant decreases in EROD, indicating an alteration of the enzyme (4). Production of the other ROS, H_2O_2, was proportional to the sum of CYP1A1 + CYP2B1/2.

Superoxide Dismutase. At the 24-month time point, SOD activities showed limited increases in response to increases in cytosolic superoxide; but correlation studies indicated that this had no more than a minor effect on tumor yields.

Glutathione Peroxidase. GPx activities showed considerable interanimal variations, but were generally strongly negatively correlated with cytosolic $\cdot O_2^-$ production and tumor yield, dropping to zero for the female 100 ppm Aroclor 1254 dose group, where the incidence of liver tumors was the highest (56%).

Glutathione. It is possible that our samples lost some GSH because of the length of sample storage time at -70 °C; nevertheless, the data obtained presented no evidence that the GSH levels in the highly tumorigenic dose groups were significantly different from those in the controls.

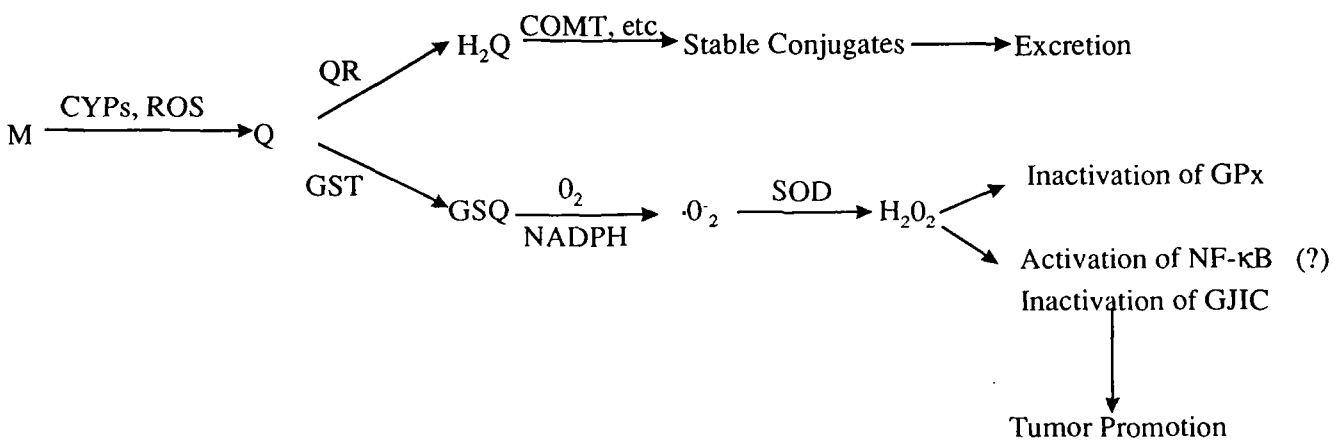
Glutathione S-Transferase. Observed levels of GST activity, unlike those of the CYP1A, CYP2B or BROD-producing Phase 1 enzymes, but like those of microsomal or cytosolic ROS production, were greater in the females. GST levels were 2-6X higher than control values in the more tumorigenic dose groups and positively correlated with tumorigenicity.

Other Enzymes. Quinone reductase, QR, also known as DT-diaphorase, is another Phase 2 enzyme that can be induced via either the Ah- or electrophile-response receptors. Its observed activities increased with those of GST and EROD in dose groups exhibiting limited GST induction (e.g., up to 2-fold) but no further in those where GST was more highly elevated. Thus, the GST/QR ratio was also positively correlated with tumor yield. A third Phase 2 enzyme, catechol 0-methyl transferase, COMT, appeared little affected by PCB dosing.

Discussion

A mechanistic model that fits the available data begins with the conversion of some unidentified metabolite(s), M, by certain of the inducible CYPs (or the microsomal ROS derived therefrom) into quinonoid oxidation product(s), Q, which may either undergo QR-mediated reduction to products that can be stabilized by methylation or other conjugation, or else undergo GST-mediated glutathionylation to give product(s), GSQ, that are no longer reactive with macromolecular nucleophiles. These GSQ, however, are still capable of redox cycling between NADPH and O_2, with resultant formation of cytosolic $\cdot O_2^-$ and H_2O_2, inactivation of GPx, and activation of responsive transcription factors, such as NF-κB (5), and inhibition of GJIC (6).

Figure 1. Proposed Roles of CYP, QR, COMT, GST, SOD, and GPx Enzymes in Production and Control of Tumorigenic Oxidants in PCB-dosed Rats' Livers



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