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The Induction of Oxidizing Agents in S-D Rats Chronically Dosed with Aroclors 1016, 1242, 1254 and 1260.

Kenneth M. Fish, Jay B. Silkworth and John F. Brown Jr.

General Electric Corporate Research and Development, PO Box 8, Schenectady, NY. 12301-0008 USA

Abstract

Basic biochemical analyses of hepatic tissues from a comparative toxicology and tumorigenicity study of four PCB compositions in Sprague Dawley rats of both sexes indicated a lack of correlation between phase one enzyme induction and tumorigenic activity; weak correlations of tumorigenicity with lipid peroxidation, as measured by TBARS, or with porphyrin accumulation; and good correlations with cytosolic NADPH oxidase activity, as measured by superoxide production. These results are consistent with the involvement of reactive oxygen species in tumorigenicity.

Introduction

In a recent chronic toxicity/carcinogenicity study[1,2] Sprague-Dawley rats were fed Aroclors 1016, 1242, 1254, or 1260 at concentrations ranging from 25-200 ppm for two years. In that study, the female rats were found to be significantly more susceptible to hepatic tumors than were the males, and the observed tumor counts were both Aroclor- and dose-dependent. A proposed sequence of events leading to tumors is being discussed by Brown et al. concurrently. In an effort to establish the biochemical basis for PCB tumor promotion in rats, we investigated the basic biochemistry of frozen hepatic tissues from these test animals.

We began with measurements of CYP 1A1 and CYP 2B1/2 protein induction; EROD, MROD, PROD and BROD activities; and TBARS (thiobarbituric acid-reactive substances) and porphyrin accumulations in order to assess the induction of phase one enzymes, their activities, and their abilities to cause oxidative stress, respectively.

Since oxidative stress appeared to be the most promising correlate of tumorigenicity, we then proceeded to make measurements of its potential sources, such as microsomal and cytosolic production of peroxide and superoxide. The results of all these assays are summarized below.

Results and Discussion

The basic biochemical findings. At 26 weeks, the males exhibited about twice the expression of hepatic microsomal CYP1A1 and CYP2B1/2 proteins and twice the induction of associated EROD, MROD, PROD and BROD activities exhibited by the females. In contrast, the females had twice the hepatic TBARS (a measure of lipid peroxidation) and ten times the hepatic porphyrins accumulated by the males.

By 52 weeks, hepatic CYP1A1 had sharply decreased in both sexes, particularly in animals dosed with Aroclors 1016 and 1260, while CYP2B1/2 levels had increased by as much as four-fold. The ratios of enzymatic EROD activities to measured CYP1A1 protein levels were reduced only in the females. Hepatic porphyrins were unchanged from levels at 26 weeks.

By 78 weeks, the expressions of CYP1A1 protein had decreased further in the males while those of CYP2B1/2 proteins decreased in both sexes. The ratios of CYP1A1 enzymatic activities to measured CYP 1A1 protein ratios in females were reduced to a greater extent than at 52 weeks and were somewhat lower in the males. By this time, the TBARS and hepatic porphyrins of the males had increased to levels similar to those of the females.

These findings showed that the rats' biochemical responses to chronic Aroclor dosing were dynamic, Aroclor-dependent and sex-dependent. The gender differences in hepatic tumorigenic response were found to be weakly correlated with those in lipid peroxidation, early porphyrin accumulation, and P450 alteration but not with CYP protein levels or their activities.

The evaluation of oxidative stress. The weak correlation of tumorigenic response with lipid peroxidation (a consequence of oxidative stress), porphyrin accumulation (which can be explained by elevated oxidant levels and inhibition of UROD) and with CYP1A1 alteration suggested possible elevations in the levels of agents that could lead to production of hydrogen peroxide and superoxide. Accordingly, we probed both the microsomal and soluble fractions of the rat livers for such activities.

The liver microsomes were indeed found to have NADPH oxidase activity, yielding peroxide and superoxide, as has been reported for similar systems[3,4]. The rates of hydrogen peroxide production were directly proportional to CYP1A1 plus CYP2B1/2 levels for both males and females at 26, 52 and 78 weeks. In contrast, the rates of superoxide production were proportional to CYP1A1 levels in males only at 26-52 weeks and females only at 26 weeks. In the males at 78 weeks and the females at 52 and 78 weeks, excess NADPH oxidase activity (superoxide production) was observed in microsomes from many of the rats dosed with 100 ppm Aroclor 1242 or 25, 50 or 100 ppm Aroclor 1254. The elevated levels of this reactive oxygen species correlated well with decreased EROD activity per μg of CYP1A1 at these time points, indicating that some change in the CYP1A1 molecule has occurred.. This alteration resulted in decreased monooxygenase activity and increased NADPH oxidase activity.

Previously, Butterworth et al.[5] showed that the estrogen quinone glutathione conjugates, produced by estradiol metabolism in hepatic microsomes, could cause redox cycling, thus producing superoxide. In addition, Amaro et al.[6] showed that PCBs could be activated to quinones that were susceptible to conjugation, while McLean et al.[7] showed that PCB quinones can also redox cycle. It follows that PCB quinones could be susceptible to the same glutathione conjugation as estrogen and that the resulting soluble conjugates could redox cycle. Therefore at least two groups of water-soluble quinones in hepatic tissues may be capable of redox cycling.

We found that the re-oxidized low molecular weight (<10 KD) soluble hepatic fractions from rats dosed with Aroclors for 18 or 24 months would redox cycle in the presence of NADPH and liver microsomes from phenobarbital-induced rats. The rates of superoxide production were greater in females than in males, in both sexes being dose- and Aroclor-dependent. The Aroclor dependence was Aroclor 1254>1260>1242>1016 in the females and Aroclor 1260>1254>1242~ 1016 for the males. The gender difference suggested that the predominant catalysts for the redox cycling could be conjugated PCB quinones in the males and conjugated estrogen quinones in the females, but this hypothesis remains to be tested. The correlation between cytosolic redox cycling and tumor counts after 24 months of dosing is shown in Figure 1.

In summary, we have found that both the microsomal and soluble fractions of the livers of Aroclor-dosed rats are sources of reactive oxygen species. The microsomal fractions contain NADPH oxidase activities that can produce both hydrogen peroxide and superoxide. The hydrogen peroxide was formed at rates proportional to the combined concentrations of CYP1A1 plus CYP2B1/2. The superoxide was produced both from native CYP 1A and in excess from altered CYP 1A, though the mode of this alteration is not yet clear. The soluble liver fractions were found to contain low molecular weight materials that were capable of redox cycling in the presence of microsomes. This activity was both dose- and Aroclor-dependent and correlated with Aroclor tumorigenicity in both sexes of the S-D rats.

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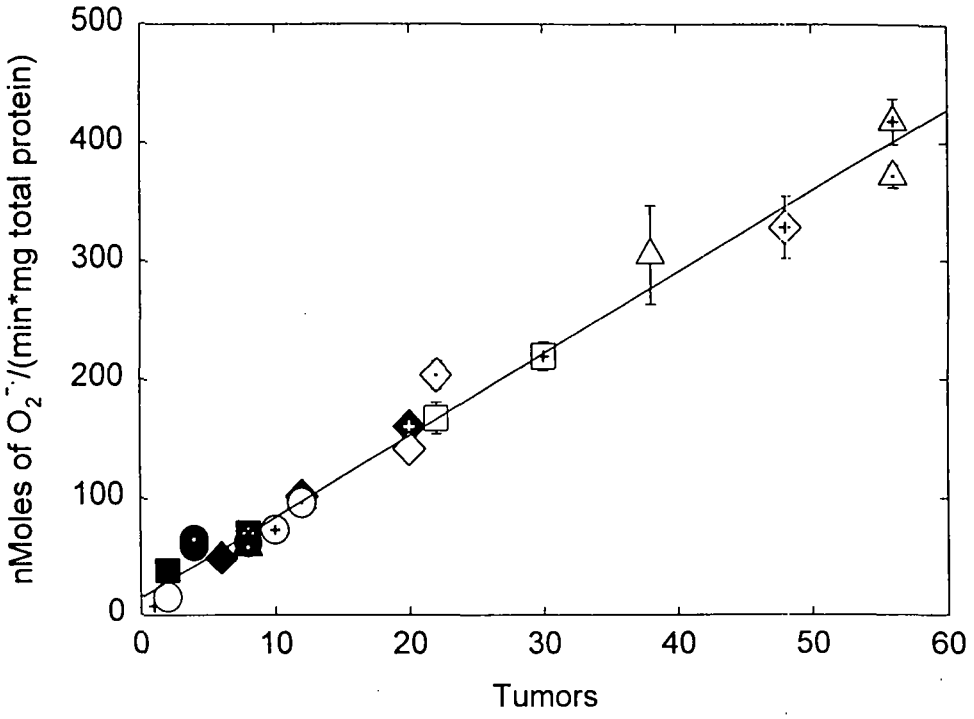
Figure 1. The Rate of Redox Cycling in Low Molecular Weight Fractions of Soluble Hepatic Tissues as a Function of Percent Rats with Tumors.



	Males				Females			
Dose (ppm)	1016	1242	1254	1260	1016	1242	1254	1260
0	+				+			
25			▲	◆			△	◇
50	●	■	▲	◆	○	□	△	◇
100	●	■	▲	◆	⊙	⊞	△	◇
200	⊕				⊕			