Biochemical and Histological Precursors of Liver Tumors in PCB-dosed Sprague-Dawley Rats

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Abstract

Detailed studies of the comparative toxicology and tumorigenicity of four PCB compositions in S-D rats of both sexes indicate the intermediate steps on the pathway to hepatotumorigenicity to include PCB accumulation in the liver, development of oxidative stress, and promotion of eosinophilic foci. There are at least two important mechanisms for the development of the oxidative stress: one TEQ-dependent and predominant in the females of this strain, the other TEQ-independent, and predominant in the males.

Introduction

The polychlorinated biphenyls (PCBs) are representative of a group of substances that are clearly non-genotoxic, yet nevertheless, when bioassayed in *ad libitum*-fed rodents near the maximum tolerated dose (MTD), can be hepatotoxic, tumor promoting, and hepatotumorigenic. There is continuing controversy as to whether such agents pose cancer risk to man. Resolution of this controversy will require identification of their mode of action as hepatotumorigens in heavily dosed rodents, and determining whether that process also operates in humans.

In hopes of producing the necessary mechanistic information, we have been collecting data on the progression of biochemical and histological changes that occurred in the livers of male and female Sprague-Dawley (S-D) rats during the course of recently completed parallel 24-month multi-dose bioassays[1,2] of Aroclors 1016, 1242, 1254, and 1260. These four formerly commercial PCB compositions have overlapping ranges of chemical composition, yet each was found to produce a somewhat different pattern of biochemical and histological changes in the livers of the two sexes. Accordingly, we have been attempting to determine which of the many observed early or mid-life changes in these parameters could be correlated with late life tumor incidence for all dose groups, and hence be implicated as likely steps in the tumorigenic process.

Results

Correlations with PCB and TEQ Accumulation: Liver tumor incidence among the Aroclor-dosed male S-D rats was significantly increased over that in the controls only for the Aroclor 1260 high dose group[1,2]. Among the other dose groups, there was a significant

correlation of tumor incidence with the time-averaged adipose (or liver) accumulation of total PCB, but not with that of dioxin toxic equivalency (TEQ). Conversely, among the female S-D rats liver tumor incidence was significantly increased in all groups except the Aroclor 1016 low dose group[1,2]. The observed tumor incidences were a little higher than those in the males for Aroclors 1016 and 1260, and much higher for Aroclors 1242 and 1254, with the increased incidences well correlated with hepatic TEQ. We concluded that there must be at least two mechanisms for hepatotumorigenesis in PCB-dosed rats: one TEQ-dependent and predominant in the S-D females, and one TEQ-independent and predominant in the males.

Correlations with Cytochrome P450 Induction: The inductions of hepatic microsomal P450 proteins and the associated enzymatic activities were generally about twice as great in the males as in the females, but showed similar dependencies on Aroclor type, dose, and time in both sexes. At six months, inductions of CYP1A1 protein and EROD and MROD activities were near maximal for all Aroclors and doses, indicating dose-saturation (and hence TEQ-independence), while the inductions of CYP 2B1/2 protein and PROD and BROD activities were all low. By 12 months, CYP1A1, EROD, and MROD activities in the Aroclor 1016- and 1260-dosed animals had dropped sharply, while remaining elevated in those dosed with Aroclor 1242 or 1254, thus restoring correlability with hepatic TEQ. Meanwhile CYP2B1/2, PROD, and BROD surged to high levels in the 1016- or 1260-dosed animals, but increased only slightly in the others. At 18 months, all levels and activities were slightly lower than at 12 months, but exhibited the same general dependencies on Aroclor type. Thus, mid-life induction of CYP1A isozymes could be correlated with hepatotumorigenicity in the females but not in the males, and induction of CYP2B isozymes did not appear to contribute appreciably to tumorigenicity in either sex.

Other Correlations: Apparent correlability with mid-life hepatic TEQ and CYP1A induction in both sexes, but with tumorigenicity only in the females, was found for the reported scorings[1,2] for hepatic hypertrophy, hyperplasia, vacuolization, and clear cell foci, and for our assays for microsomal superoxide production. CYP1A-correlated changes observed primarily in the females included sinusoidal pigmentation and increases in hepatic iron and selenium, as well as in hepatotumorigenicity. Fair correlations with tumorigenicity for both sexes were seen for the incidence of eosinophilic foci[1,2], and the extents of oxidation of lipids (as measured by TBARs) and oxidation of porphyrinogens (as measured by porphyria). These latter observations were unsurprising. Eosinophilic foci in rat livers have also been observed following chronic dosing with either phenobarbital or dioxin in the absence of an initiating dose of a mutagen. (By contrast, the foci promotable after such initiation are basophilic). All other classes of substances indicated as "tumor promoters" by either the classic mouse skin or rat liver two-stage bioassays also appear to be sources of oxidative stress, albeit by a variety of mechanisms[3,4]. It would thus appear that the development of oxidative stress and the promotion of spontaneously initiated eosinophilic foci may be intermediate steps in the pathway between PCB ingestion and hepatotumorigenesis for rats of both sexes.

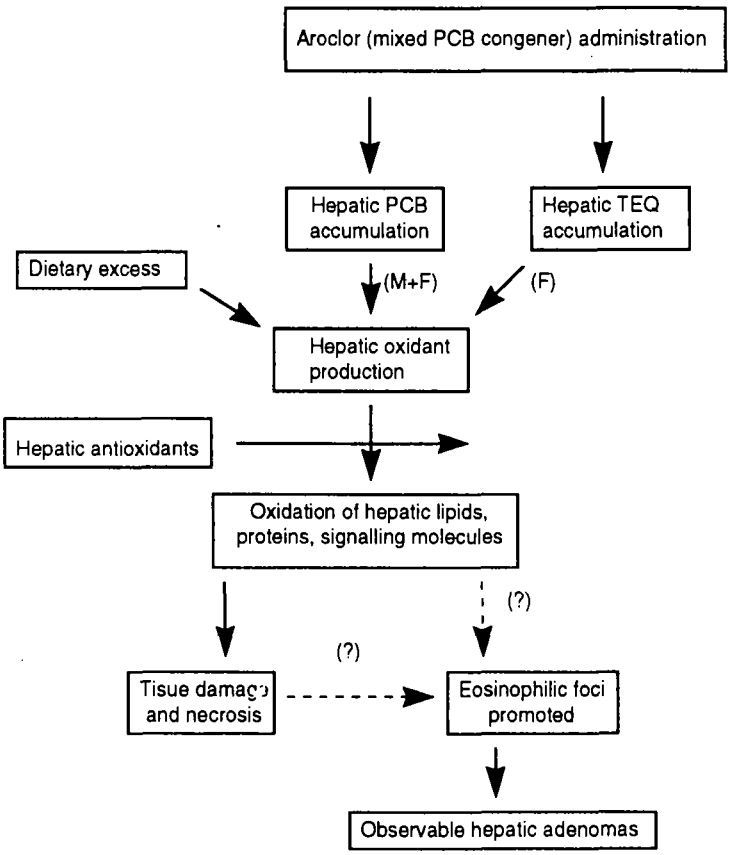
Alternative Sources of Oxidative Stress: Oxidative stress, as indicated by the oxidation of lipids, proteins, and other intracellular biochemical species, presumably occurs when the production of free radical and other oxidants exceeds that of intracellular antioxidants. Observed or reported sources of hepatic oxidant production in PCB/rat bioassay systems include: (a) mitochondrial superoxide production, primarily in response to the high caloric intake resulting from the routinely used *ad libitum* feeding protocols[5]; (b) microsomal superoxide production, which we found to correlate with CYP1A1; (c) microsomal H_2O_2

production, which we found to correlate with CYP1A1 and CYP2B1/2; (d) superoxide production by PCB-stimulated hepatic neutrophils, a process reported to be mediated by phospholipase A_2 rather than a P450[6]; and (e) cytoplasmic superoxide production by redox cycling of quinones, such as those formed by the CYP1A-mediated oxidation of estrogen catechols and subsequent conjugation with glutathione[7,8].

Measurements of the redox cycling activities of the soluble, low molecular weight fractions of the bioassayed rat livers, reported elsewhere at this meeting by KM Fish *et al.*, were found to correlate with tumor incidence in both sexes, implying significant participation by source (e).

Discussion

The correlations thus far observed suggest that the overall sequence of events leading to liver tumors in Aroclor-dosed male (M) or female (F) S-D rats may be as diagrammed below, with specific mechanistic details of the oxidant production and foci promotion processes still to be determined.



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