Effect of Dietary Antioxidants on the Promotion of Hepatocarcinogenesis by PCBs

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Introduction

Mixtures of halogenated biphenyls as well as many individual congeners have been reported to be promoters of carcinogenesis in various liver tumor models[1]. However, their mechanism of action is not known. A number of mechanisms have been investigated, including direct effects on signal transduction pathways, induction of oxidative stress, effects on vitamin A metabolism, and effects on intercellular communication[1]. One mechanism by which PCBs may promote hepatic tumors is by inducing oxidative damage in the liver. Forms of oxidative damage that may be important are the induction of lipid peroxidation, the induction of oxidative DNA damage, and the alteration of gene expression. One possible mechanism for inhibiting the promoting activity of PCBs may be to increase the concentration of antioxidants in the diet. In this study, we examined if dietary selenium or antioxidant phytochemicals could inhibit the hepatic promoting activity of PCBs in rats.

Methods and Materials

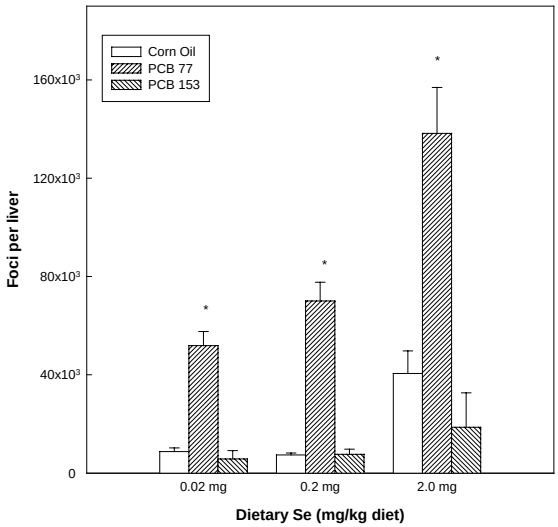
Female Sprague Dawley rats were obtained from Harlan Sprague Dawley (Indianapolis, IN). All the animals in these studies received a single dose of diethylnitrosamine (DEN, 150 mg/kg). In all studies, rats received four biweekly injections of 3,3',4,4'-tetrachlorobiphenyl (PCB-77) or 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153) (300 μmol/kg). Ten days after the last PCB injection, all animals were sacrificed; 3 days before sacrifice all animals were implanted with Alzet osmotic pumps containing 5-bromo-2'-deoxyuridine (BrdU). Paraffin sections were double immunostained for placental glutathione S-transferase (PGST) and BrdU. The number and volume of PGST-

positive foci were quantified on a computer digitizing system, as described previously [2-4]. The rate of hepatic DNA synthesis was estimated by quantifying labeling indexes of BrdU-stained nuclei.

We first examined the effect of dietary selenium on the hepatic tumor promoting activity of PCB-77 and PCB-153 in female Sprague-Dawley rats. One week after DEN injection, rats were fed purified diets containing 0.02, 0.2 (similar to the 0.15 ppm recommended for rodent diets[5]), or 2.0 ppm selenium in the form of sodium selenite. Starting one week later, we injected rats i.p. with vehicle (corn oil), PCB-77 or PCB-153 (300 µmol/kg) every 14 days for 4 injections. All rats were euthanized 10 days after the last PCB injection.

We next examined the effect of non-nutritive phytochemicals. One week after DEN injection, rats were fed a control purified diet or the same diet containing ellagic acid (0.4%), beta-carotene (0.5%), curcumin (0.5%), N-acetyl cysteine (NAC, 1.0%), Coenzyme Q_{10} (CoQ_{10}; 2.0%), resveratrol (0.005%), lycopene (10% as LycoVit [BASF]), which contains 10% lycopene), and epigallocatechin-3-gallate (EGCG, 1% as a green tea extract containing 16.5% EGCG and 33.4% total catechins). Rats were fed the diets for the remainder of the study. After 3 weeks, 2/3 of the control rats and all of the rats receiving the phytochemicals were injected i.p. with PCB-77 (300 µmol/kg) every 14 days for 4 injections. All rats were euthanized 10 days after the last PCB injection.

Figure 1A. Effect of Selenium of the No. of Foci per Liver

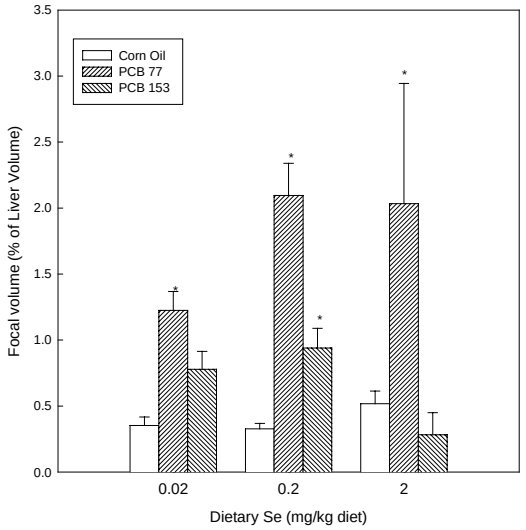


Results and Discussion

In these studies, we examined whether dietary antioxidants would inhibit the promoting activities of PCBs. We examined a nutritive antioxidant, selenium, as well as several non-nutritive antioxidants. Selenium is a component of several of the isozymes of glutathione peroxidase (GPx), which metabolizes hydrogen peroxide, as well as of other proteins, such as selenoproteins P and W, thioredoxin reductase, and 5'-iodothyronine deiodinase[6].

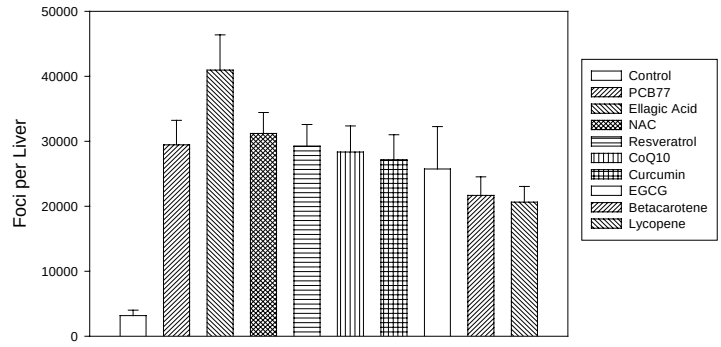
We first examined the effect of dietary selenium on the hepatic tumor promoting activity of PCB-77 and PCB-153 in female Sprague-Dawley rats. As observed previously[1], PCB-77 increased the number and volume of PGST-positive foci. Increasing the amount of dietary selenium from 0.02 to 0.2 ppm was found to increase the number and volume of PGST-positive foci in PCB-77-treated rats; further increasing selenium from 0.2 to 2.0 ppm further increased the number of foci per liver, but had no effect on focal volume (**Figure 1**). PCB-153 increased the volume but not the number of foci at the 0.02 and 0.2 ppm Se levels, but actually slightly decreased the number and volume of foci at the 2.0 ppm Se level. Therefore, dietary selenium does not inhibit the induction of PCB-77 induced foci; rather the recommended level appears to be required for optimum foci development.

Figure 1B. Effect of Selenium on Focal Volume



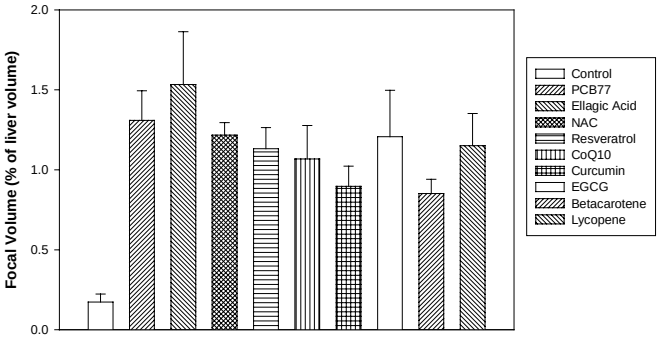
We have also examined non-nutritive phytochemicals to determine if they can inhibit the tumor promoting activity of PCBs in rats. We focused on antioxidant phytochemicals: lycopene, epigallocatechin-3-gallate (EGCG), coenzyme Q_{10} (CoQ_{10}), curcumin, resveratrol, β-carotene, N-acetyl cysteine (NAC), ellagic acid, and butylated hydroxyanisole (BHA). Rats receiving PCB-77 had a large increase in PGST-positive foci compared to those not receiving PCB-77. Three antioxidants inhibited the induction of foci: lycopene decreased the number of foci; curcumin decreased the size of the foci; and β-carotene decreased both the number and size of the foci (**Figure 2**). Ellagic acid

Figure 2A. Effect of Antioxidant Phytochemicals on the Number of Foci per Liver



increased both the number and volume of PGST-positive foci. The other antioxidants had minor effects.

Figure 2B. Effect of Antioxidant Phytochemicals on Volume Fraction of Foci



Several of these agents appear to increase the promoting activity of PCBs or to have no effect: e.g. selenium, ellagic acid, N-acetyl cysteine (NAC), and resveratrol. Previously we found that dietary vitamin E also did not affect the promoting activities of PCBs[7]. In contrast, others appeared to inhibit the promoting activity of PCBs: β-carotene, curcumin, and lycopene. In addition, we previously found that vitamin A (in the form of retinyl palmitate) inhibited the promoting activity of both PCB-77 and PCB-153[8]. One of the inhibitory antioxidants, β-carotene, has vitamin A activity; therefore it could be acting as an antioxidant and/or through its vitamin A activity. PCBs in several studies have also been shown to decrease hepatic levels of vitamin A[1]. In addition, the retinoids are involved in cellular growth and differentiation. Their effects are mediated by two families of receptors: the retinoic acid receptors (RAR) and the retinoid X receptors (RXR), each of which have three subtypes[9]. Both of these receptors, after activation by ligands (retinoic acid for RAR and 9-cis-retinoic acid for RXR), function as transcription factors. In addition to forming homodimers or heterodimers with each other, these receptors can also form heterodimers with several other transcription factors. Therefore, the alteration of vitamin A metabolism by PCBs could have profound effects on gene expression in the cell.

Acknowledgements

This work was supported by a grant from the Superfund Basic Research Program, National Institute of Environmental Health Sciences (ES07380).

References

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