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ROLE OF OXIDATIVE STRESS IN THE PROMOTING ACTIVITIES OF PCBS

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Abstract

PCBs are organic pollutants that persist and bioaccumulate in the environment. These chemicals induce and promote liver tumors in rodents. Previous studies have shown that they increase oxidative stress in the liver, including lipid peroxidation, oxidative DNA damage, and NF-κB activation. The objective of these studies was to determine if the promoting activities of PCBs could be inhibited by dietary antioxidants (vitamin E, selenium, or phytochemicals) or by knocking out the p50 subunit of NF-κB. In the antioxidant studies, female rats were first injected with DEN (150 mg/kg) and then administered 4 biweekly i.p. injections (300 μmol/kg/injection) of PCB-77, PCB-153, or vehicle; the number and volume of placental glutathione S-transferase (PGST)-positive foci were then quantified. Vitamin E did not influence the promoting activities of PCBs. Increasing dietary selenium above the recommended intake increased the number of foci induced but decreased their volume. Most of the phytochemicals examined (N-acetyl cysteine, β-carotene, resveratrol, EGCG) had no significant effect on the promoting activity of PCB-77. Ellagic acid increased and lycopene decreased the number of foci; ellagic acid, CoQ_{10}, and curcumin decreased the volume of foci. In the NF-κB knockout study, male mice were first injected with DEN (90 mg/kg); controls not receiving DEN were also studied. Both p50 −/− and wild-type mice were then injected biweekly 20 times with PCB-153 (300 (μmol/kg). In DEN-treated and DEN + PCB-treated mice, the incidence of tumors was lower in the p50 −/− mice than in wild-type mice. In mice receiving PCB-153, the tumor incidence and tumor volume were higher. The volume of tumors that were positive for glutamine synthetase was increased in mice administered PCB-153. This study shows that the promotion of hepatocarcinogenesis by PCBs is largely unaffected by dietary antioxidants but is diminished when NF-κB activation is impaired by the absence of the p50 subunit.

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Keywords

vitamin E; selenium; phytochemicals; NF-κB; carcinogenesis

Introduction

PCBs produce a variety of biological effects, including a wasting syndrome, chloracne, immunosuppression, and hepatomegaly (Robertson and Hansen 2001). They activate specific receptors, such as the Ah receptor and the constitutive androstane receptor (CAR), and induce the specific cytochrome P-450s that are activated by these receptors (Robertson and Hansen 2001). PCB mixtures have been shown to be carcinogenic in the liver (Silberhorn et al. 1990). In addition, many PCB congeners have been demonstrated to have promoting activity; studies have found them to increase the formation of both tumors and altered hepatic foci (Glauert et al. 2001). However, the mechanisms of promotion by PCBs are not definitively known.

One of the mechanisms by which PCBs may exert their promoting activity is by increasing hepatic oxidative stress (Figure 1). PCBs have been observed in many studies to induce oxidative stress. Several studies have shown that PCBs increase lipid peroxidation in the liver (Dogra et al. 1988;Fadhel et al. 2002;Kamohara et al. 1984;Oda et al. 1987;Pelissier et al. 1990;Saito 1990;Shara and Stohs 1987;Yamamoto et al. 1994). PCBs also can produce oxidative DMA damage, in the form of 8-hydroxydeoxyguanosine (Oakley et al. 1996). We have observed that PCBs can activate NF-κB, which may be activated by oxidative stress (Li and Karin 1999;Schreck et al. 1992;vandenBerg et al. 2001). We examined two PCBs: 3,3′ 4,4′-tetrachlorobiphenyl (PCB-77), an Ah receptor agonist; and 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB-153), a CAR activator. PCB-77 did not increase the hepatic DNA binding activity of NF-κB in short-term studies, but did after four biweekly injections (Glauert et al. 2005;Lu et al. 2003;Tharappel et al. 2002). PCB-153, on the other hand, increased the DNA binding activity of NF-κB in short-term studies and in one of two long-term studies (Glauert et al. 2005;Lu et al. 2004;Lu et al. 2003;Tharappel et al. 2002).

We therefore hypothesized that the tumor promoting activities of PCBs could be inhibited by decreasing oxidative stress in the liver (Figure 1). We first examined if the tumor promoting activities of PCBs could be inhibited by increasing the consumption of antioxidants, including vitamin E, selenium, and several antioxidant phytochemicals such as β-carotene, lycopene, and curcumin. We then examined if inhibiting NF-κB activation by deleting the p50 subunit of NF-κB could inhibit the promoting activities of PCBs.

Vitamin E

In the first study, the effect of dietary vitamin E on the hepatic tumor promoting activities of PCB-77 and PCB-153 in female Sprague-Dawley rats (175–200 g) was investigated (Glauert et al. 2005). One week after diethylnitrosamine (DEN) injection, rats were fed purified diets containing 10, 50 or 250 mg/kg vitamin E in the form of α-tocopheryl acetate. Starting one week later, we injected rats i.p. with vehicle (corn oil), or 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. The number and volume of placental glutathione S-transferase (PGST)-positive foci were increased by PCB-77 but not by PCB-153. Vitamin E did not affect the induction of PGST-positive foci. PCB-77, but not PCB-153, caused an increase in hepatic NF-κB activity, and this increase was slightly, but insignificantly, inhibited by high-level vitamin E. Therefore dietary vitamin E supplementation did not protect against the induction of altered hepatic focal lesions by PCBs.

Selenium

We next examined the effect of dietary selenium on the tumor promoting activities of PCB-77 and PCB-153. We hypothesized that the promoting activities of PCBs would be inhibited, since selenium is a component of the antioxidant enzymes glutathione peroxidase and thioredoxin reductase (Rayman 2005; Tapiero et al. 2003). An AIN-93 torula-based purified diet containing 0.02 (deficient), 0.2 (adequate), or 2.0 mg (supplemental) selenium/kg diet was fed to Sprague-Dawley female rats starting ten days after administering a single dose of DEN (150 mg/kg). Four i.p. injections of either PCB-77 or PCB-153 (300 (μmol/kg, administered every 14 days) were given to the rats after 3 weeks on the selenium diet. The total number of PGST-positive foci in the liver and the number of PGST-positive foci per cubic centimeter of liver among the PCB-77 treated rats were increased as the dietary level of selenium increased. Unlike PCB-77, PCB-153 did not show the same selenium dose-response effect; nevertheless, selenium supplementation did not confer protection against the number of foci induced. However, the 2.0 ppm selenium diet reduced the mean focal volume, indicating a possible protective effect by inhibiting progression of preneoplastic lesions into larger foci. Cell proliferation was not inhibited by selenium in the liver of the PCB-treated groups. Selenium did not prevent the PCB-77 induced decrease of hepatic selenium and associated reduction in glutathione peroxidase (GPx) activity. In contrast, thioredoxin reductase (TrxR) activity was not affected by the PCBs treatment or by selenium supplementation. These findings indicate that selenium does not inhibit the appearance of PGST-positive foci during promotion by PCBs, but that the growth of the lesions may be inhibited. The effects of selenium on altered hepatic foci did not correlate with its effects on GPx and TrxR.

Antioxidant Phytochemicals

To conclude our antioxidant studies, we examined the effects of several antioxidant phytochemicals on the tumor promoting activity of PCB-77. Female Sprague Dawley rats were first injected i.p. with DEN (150 mg/kg) and one week later the rats were fed an AIN-93 based purified diet or the same diet containing ellagic acid (0.4%), beta-carotene (0.5%), curcumin (0.5%), N-acetyl cysteine (MAC) (1.0%), CoQ_{10} (2.0%), resveratrol (0.005%), lycopene (10% as LycoVit, which contains 10% lycopene), or epigallocatechin-3-gallate (EGCG; a 1% 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 and all of the antioxidant diet-fed rats were injected i.p. with PCB-77 (300 μmol/kg) every 14 days for four injections. All rats were euthanized 10 days after the last PCB injection. The rats that received PCB-77 alone showed a large increase in placental glutathione S-transferase (PGST)-positive foci in the liver. Lycopene decreased the number of foci, while curcumin and CoQ_{10} decreased the size of the foci. Beta-carotene slightly decreased both the number and size of the foci. In contrast ellagic acid significantly increased the number but decreased the size of the foci. All of the other phytochemicals showed only minor effects. These findings show that some antioxidant phytochemicals may inhibit the promoting activity of PCB-77, but that most show no effect.

NF-κB

We next examined if NF-κB is necessary for PCB-mediated changes in cell proliferation and apoptosis, and for the promoting activities of PCBs. We used a mouse model that is deficient in the p50 subunit of NF-κB (p50 −/−) for these studies (Sha et al. 1995). We first hypothesized that cell proliferation in response to PCB-153 would also be altered in p50 −/− mice (Lu et al. 2004). In a two-day study, male wild-type and p50 −/− mice received a single i.p. injection of corn oil or PCB-153 (300 μmol/kg) and were euthanized after 48 hours. In a 21-day study, mice received six i.p. injections of corn oil or PCB-153 (100 μmol/kg) between days 1 and 17 and were euthanized 4 days after last injection. Nuclear NF-κB DNA binding activity,

measured by electrophoretic mobility shift assay, was present in control wild type mice and increased with PCB-153 in the two-day study. NF-κB DNA binding activity was very low in the p50−/− mice and did not increase with PCB-153. Hepatocyte proliferation was measured in these mice by the BrdU labeling index. Control wild type mice had the same level of cell proliferation as the control p50−/− mice. In the two-day study, hepatic NF-κB DNA binding activity and cell proliferation were increased by PCB-153 in wild-type but not in p50−/− mice. In the 21-day study, cell proliferation was significantly increased in the wild-type mice treated with PCB-153; in the p50 −/− mice treated with PCB-153, cell proliferation was increased compared to untreated mice but less than wild-type mice treated with PCB-153. Apoptosis in hepatocytes was measured by the TUN EL assay. The p50 −/− mice had more spontaneous apoptosis than wild-type mice, and PCB-153 inhibited apoptosis in p50 −/− mice (for both time points). These data indicate that NF-κB is involved in the proliferative and apoptotic changes in response to PCB-153.

We hypothesized that gene expression related to cell proliferation and apoptosis would be altered in p50 −/− exposed to PCB-153 (Lu et al. 2004). Since PCB-induced cell proliferation was inhibited in p50 −/− mice, we hypothesized that the expression of cell cycle genes would be altered. The cell cycle control system is regulated by the activity of cyclins, cyclin-dependent kinases (CDKs) and cyclin-dependent kinase inhibitors (CKIs) (Johnson and Walker 1999). The mRNA levels of cyclins A2, B1, B2, C, D1 and D2 in liver were measured using RNase protection assays (RPAs). For the cyclins analyzed, there were no significant differences between wild type mice and p50−/− mice at either time point. PCB-153 significantly decreased cyclin A2, B1, B2, and C mRNA levels in the two-day study in both p50−/− and wild-type mice, but not that of cyclin D1 or D2; in the 21-day study, the mRNA levels of all cyclins were not significantly different among groups. The cyclin D1 levels were measured using Western blotting. Compared to wild-type mice, p50−/− mice had less cyclin D1 protein in the liver. However, cyclin D1 protein levels in the liver of both groups of mice were not changed by PCB-153 at either timepoint. Therefore, we could not identify the NF-κB -regulated genes that were important in the induction of cell proliferation by PCB-153 from these studies. We also examined if the signal transduction pathway leading to NF-κB activation was altered differently by PCBs in wild-type or p50 −/− mice. The protein levels of IKKs (IKKα, IKKβ, IKKγ) and IκBβ were not changed by PCB treatment, and there was no difference in these proteins between wild type mice and p50−/− mice. There was less IκBα protein in p50−/− mice than in wild types, but IκBα protein was not affected by PCB-153 treatment.

We next examined the promotion of hepatocarcinogenesis by PCB-153 in the p50 knockout (p50 −/−) mice. Since the induction of cell proliferation by PCB-153 was inhibited in p50 −/− mice, we hypothesized that the deletion of the p50 subunit would inhibit the promoting activities of PCBs. We previously observed that the promoting activity of the peroxisome proliferator Wy-14,643 was inhibited in p50 −/− mice (Glauert et al. 2006). Wild type and p50 −/− male mice were injected with DEN (90 mg/kg) and then subsequently received 20 biweekly injections of PCB-153. Controls not receiving DEN were also studied. In DEN-treated mice, p50 −/− mice had a lower incidence of tumors. PCB treatment did not significantly affect the tumor incidence, although mice treated with PCB-153 had a slightly higher incidence. The volume of tumors in DEN-treated mice was quantified using glutamine synthetase (GS) as a marker; both GS-positive and GS-negative tumors were identified. The volumes of total and GS-positive tumors were increased by PCB-153. Tumors that were positive for GS were also found to have mutations in the β-catenin gene (Strathmann et al. 2006). The volume of GS-positive tumors was decreased by p50 deletion in control and PCB-153 treated mice. The volume of GS-negative tumors, however, was significantly increased by p50 deletion. In DEN-treated mice, cell proliferation in normal hepatocytes was significantly increased in mice receiving PCB-153; this increase was inhibited in p50 −/− mice. In hepatic tumors, the rate of cell proliferation was much higher than in normal hepatocytes, but was not affected by PCB

treatment or p50 deletion. The rate of apoptosis, as measured by the TUNEL assay, was slightly lower in mice receiving PCB-153. In hepatic tumors, the rate of apoptosis was lower than in normal hepatocytes, but was not affected by PCB treatment or p50 deletion. Overall, deleting the p50 subunit of NF-κB appears to decrease the promoting activity of PCB-153.

Conclusions

In summary, the results from these studies do not support the hypothesis that increasing dietary antioxidants can inhibit the tumor promoting activities of PCBs. Increasing the consumption of selenium did appear to inhibit the growth of foci, and some of the antioxidant phytochemicals did inhibit the number or volume of foci, but overall the dietary antioxidants did not have a dramatic effect. On the other hand, inhibiting the activation of NF-κB by the deletion of the p50 subunit decreased the promoting activity of PCB-153. Examining effects on hepatic gene expression in NF-κB knockouts may therefore provide clues to the mechanisms by which PCB-153 is promoting hepatic tumors.

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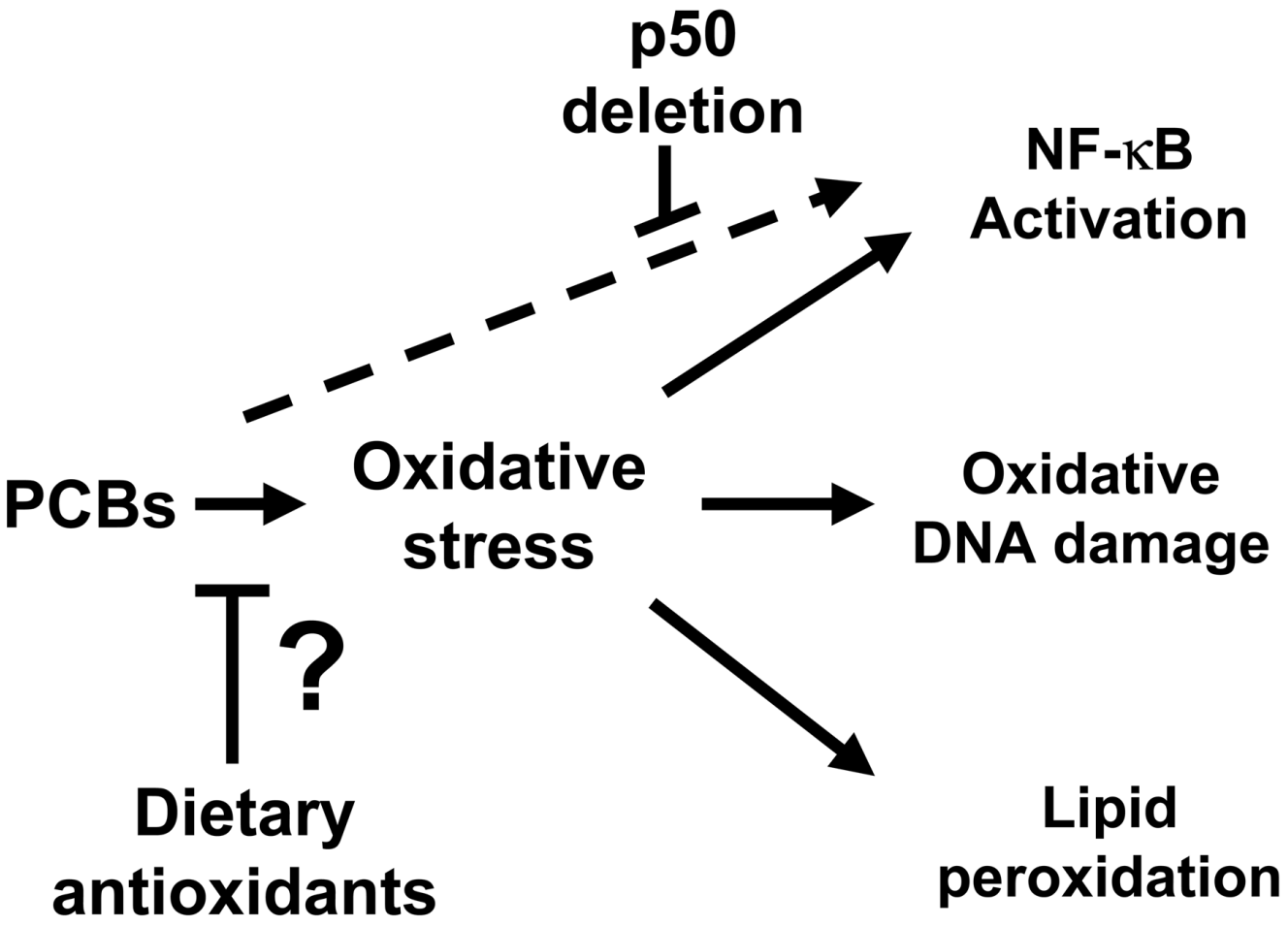

Figure 1. Mechanisms by which PCB-induced oxidative stress may influence hepatic tumor promotion

PCBs may exert their hepatic tumor promoting activities by increasing oxidative stress. PCBs have previously been shown to increase hepatic lipid peroxidation, oxidative DNA damage, and NF-κB activation, all of which can be produced by oxidative stress. NF-κB activation may also possibly be increased by PCBs independently of oxidative stress. We hypothesized that the tumor promoting activities of PCBs may be inhibited by increasing dietary antioxidants or by deleting the p50 subunit of NF-κB.