Published in final edited form as:
Food Chem Toxicol. 2008 November ; 46(11): 3467–3474. doi:10.1016/j.fct.2008.08.023.

Effect of Antioxidant Phytochemicals on the Hepatic Tumor Promoting Activity of 3,3′,4,4′-Tetrachlorobiphenyl (PCB-77)

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Abstract

Polychlorinated biphenyls (PCBs) have promoting activity in the liver, which may be brought about in part by the induction of oxidative stress. In this study we examined the effects of several antioxidant phytochemicals on the tumor promoting activity of 3,3′,4′4-tetrachlorobiphenyl (PCB-77). Female Sprague Dawley rats were first injected with diethylnitrosamine (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%), β-carotene (0.5%), curcumin (0.5%), N-acetyl cysteine (NAC, 1.0%), co-enzyme CoQ_{10} (CoQ_{10}, 0.4%), resveratrol (0.005%), lycopene (10% as LycoVit (Sweeny *et al.*), which contains 10% lycopene), or a tea extract (1%, containing 16.5% epigallocatechin-3-gallate [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 antioxidant diet-fed rats were injected i.p. with PCB-77 (300 μmol/kg) every other week for four injections. All rats were euthanized 10 days after the last PCB injection. The rats that received PCB-77 alone showed an increase in the number and size of placental glutathione S-transferase (PGST)-positive foci in the liver. Lycopene significantly decreased the number of foci, while curcumin and CoQ_{10} decreased the size of the foci. In contrast ellagic acid increased the number but decreased the size of the foci. All of the other phytochemicals showed only slight or no effects. Compared with the PCB-77 group, CoQ10 increased cell proliferation in normal hepatocytes, whereas the other antioxidants had no effect in either normal or PGST-positive hepatocytes. These findings show that none of the antioxidant phytochemicals produced a clear decrease in the promoting activity of PCB-77.

Keywords

antioxidant; PCB; phytochemicals; altered hepatic foci

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Introduction

Polychlorinated biphenyls (PCBs) cause several toxic effects in the liver, including the induction of hepatocellular carcinoma (Silberhorn *et al.*, 1990; van Birgelen *et al.*, 1996; Robertson and Hansen, 2001). Several PCB mixtures and certain specific PCB congeners have been shown to have promoting activity in the liver (Silberhorn *et al.*, 1990; Safe, 1994; Glauert *et al.*, 2001). The PCB mixtures and PCB congeners that have promoting activity are described in Glauert et al. (2001). The mechanisms of the promoting activity of PCBs, however, have not been determined. A number of mechanisms have been proposed, including direct effects on signal transduction pathways, induction of oxidative stress, effects on vitamin A metabolism, and effects on intercellular communication (Glauert *et al.*, 2001).

One mechanism by which PCBs may exert their promoting activity is by increasing hepatic oxidative stress. PCBs have been observed in many studies to induce oxidative stress. Several studies have shown that PCBs increase lipid peroxidation in the liver (Kamohara *et al.*, 1984; Oda *et al.*, 1987; Shara and Stohs, 1987; Dogra *et al.*, 1988; Pelissier *et al.*, 1990; Saito, 1990; Yamamoto *et al.*, 1994; Fadhel *et al.*, 2002). PCBs also can produce oxidative DNA damage, in the form of 8-hydroxydeoxyguanosine (Oakley *et al.*, 1996). We have observed that PCBs can activate NF-κB (Tharappel *et al.*, 2002; Lu *et al.*, 2003; Lu *et al.*, 2004), which is known to be activated by oxidative stress (Schreck *et al.*, 1992; Li and Karin, 1999; vandenBerg *et al.*, 2001), and that the promoting activity of 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB-153) is inhibited by the deletion of the p50 subunit of NF-κB (Glauert *et al.*, 2008).

We therefore hypothesized that the tumor promoting activities of PCBs could be inhibited by decreasing oxidative stress in the liver. The present study examines if supplementing antioxidant phytochemicals could prevent the promotion of altered hepatic foci by 3,3′,4,4′-tetrachlorobiphenyl (PCB-77). The phytochemicals selected for this study were ellagic acid, β-carotene, curcumin, N-acetyl cysteine (NAC), coenzyme Q_{10} (CoQ10), resveratrol, lycopene, and a tea extract containing epigallocatechin-3-gallate (EGCG). All of these agents have been found to inhibit experimental carcinogenesis in several studies (Wood *et al.*, 1982; Mukhtar *et al.*, 1986; Suzuki *et al.*, 1986; Toma *et al.*, 1995; Yang *et al.*, 2001; Balansky *et al.*, 2002; Nishino *et al.*, 2002; Yang *et al.*, 2002; Hannum, 2004; Joe *et al.*, 2004; Russell, 2004; Bhuvaneswari and Nagini, 2005; Perumal *et al.*, 2005; Nishikawa-Ogawa *et al.*, 2006; Sakano *et al.*, 2006; Thangapazham *et al.*, 2006). Ellagic acid is a naturally occurring plant phenol that has strong scavenging ability for hydrogen peroxide, superoxide anion and hydroxy anion in vitro (Cozzi *et al.*, 1995; Iino *et al.*, 2001). The carotenoid β-carotene has been demonstrated to quench singlet oxygen and also scavenge peroxy radicals (Paiva and Russell, 1999). Curcumin has antioxidant activity against free radicals, and also increases the activity of antioxidant enzymes (Joe *et al.*, 2004). NAC is a precursor of glutathione and increases glutathione levels; it also can scavenge free radicals itself (Zafarullah *et al.*, 2003; Aitio, 2006). NAC has also been shown to modulate transcriptional activities through pathways involving c-fos/c-jun, NF-κB, and cyclin inhibitors (Zafarullah *et al.*, 2003). CoQ10 is a component of the electron transport pathway in mitochondria (Kwong *et al.*, 2002). The quinol form of CoQ10 is a potent antioxidant in the inner mitochondrial membrane. It inhibits lipid peroxidation by either scavenging free radicals or reducing the alpha tocopheryl radical (Turrens *et al.*, 1985; Battino *et al.*, 1990; Ernster and Dallner, 1995). Resveratrol is found in grapes and other plants and is a phytoalexin (antifungal agent) with strong antioxidant activity (Fauconneau *et al.*, 1997); it has also been found to be a competitive inhibitor of Ah receptor ligands and to inhibit dioxin response element driven transcription of CYP1A1 and other phase 1 enzymes in vivo (Ciolino *et al.*, 1998; Casper *et al.*, 1999). Lycopene is one of the major carotenoid antioxidants in tomatoes and is known to have a significant anticancer effect with its singlet oxygen quenching ability (Di Mascio *et al.*, 1989; Shi *et al.*, 2004). EGCG and other polyphenols present in green tea have antioxidant activity and also prevent oxidation by

chelating metal ions such as iron or copper (Cabrera *et al.*, 2006). EGCG has also been found to modulate signal transduction pathways that inhibit cell proliferation and increase apoptosis (Khan *et al.*, 2006).

Several models have been used to study the initiation and promotion of carcinogenesis in the liver. Initiation generally consists of the administration of a necrogenic dose of an initiating agent or a non-necrogenic dose in combination with a proliferative stimulus, such as partial hepatectomy (Glauert, 1991). Promotion consists of the long-term administration of generally non-genotoxic agents such as phenobarbital, peroxisome proliferators, or PCBs, and results in the formation of hepatocellular adenomas and carcinomas (Pitot *et al.*, 1987; Glauert *et al.*, 2001). In addition, foci of putative preneoplastic hepatocytes, which have altered enzyme activities or cellular functions, appear before the development of gross tumors (Glauert, 1991).

In this experiment we investigated if the above antioxidant phytochemicals would inhibit the hepatic tumor promoting activity of PCB-77. We used PCB-77 because this PCB congener has efficacious hepatic tumor promoting activity (Glauert *et al.*, 2001). After initiation with diethylnitrosamine (DEN), rats were fed a diet containing one of these antioxidants or a control diet before receiving four biweekly injections of PCB-77. The number and volume of placental glutathione S-transferase (PGST)-positive foci were quantified, as well as the rate of cell proliferation in normal and PGST-positive hepatocytes.

Materials and Methods

Chemicals

PCB-77 was synthesized and characterized as described previously (Schramm *et al.*, 1985; Lehmler and Robertson, 2001). The purity of the PCB-77 was >99% by GC-MS. Diethylnitrosamine (DEN, catalog #N0258, purity unknown) was purchased from Sigma Chemical Company (St. Louis, MO). Anti-PGST antibody was purchased from Novocastra Laboratories (Newcastle upon Tyne, UK) and ABC and ABC-AP staining kits were purchased from Vector Laboratories (Burlingame, CA). Antigen retrieval Citra solution was obtained from Biogenex (San Ramon, CA). Alzet osmotic pumps (model 2ML1) were obtained from Alza Scientific Products (Palo Alto, CA). The anti-5-bromo-2′-deoxyuridine (BrdU) antibody was purchased from Becton-Dickinson (San Jose, CA). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

Experimental Diets

Ellagic acid, N-acetyl-L-cysteine (NAC), resveratrol, β-carotene, and curcumin were purchased from Sigma Chemical Co. (St. Louis, MO). A tea extract containing 16.5% EGCG and 33.4% total catechins, was a generous gift from Jarrow Formulas, Inc. (Los Angeles, CA). Coenzyme Q_{10} complex 20% (Co Q_{10}) was purchased from Tishcon Corp., Westbury, NY. Lycovit (containing 10% lycopene) was a generous gift from BASF Corporation (Shreveport, LA). Casein, corn starch, dextrose monohydrate, soybean oil, non nutritive cellulose fiber, AIN-93 mineral mix, AIN-93 vitamin mix, choline bitartrate and L-cystine were purchased from Harlan Teklad Test Diets, Madison, Wisconsin. The composition of the control diet, which was based on the AIN-93 diet (Reeves *et al.*, 1993), is shown in Table 1. Purified diets were used because they have lower levels of naturally-occurring phytochemicals. Purified diets consist primarily of refined ingredients with added vitamin and mineral mixtures, whereas unrefined (i.e. chow) diets are composed primarily of unrefined plant and animal materials [Bieri, 1977 #54]. The experimental diets were made by mixing in the following concentrations of the phytochemicals: ellagic acid, 0.4%; NAC, 1.0%; resveratrol, 0.005%; CoQ_{10}, 0.4% (2% of 20% CoQ_{10} complex); curcumin, 0.5%; tea extract, 1.0%; β-carotene, 0.5%; lycopene,

10.0% Lycovit. The doses chosen were based on previous studies using these phytochemicals (Daniel and Stoner, 1991; Hirose *et al.*, 1995; Lubet *et al.*, 1997; Hirose *et al.*, 1999; Schilling *et al.*, 2001; Thomas *et al.*, 2001; Martin *et al.*, 2002; Witschi *et al.*, 2002; Jonker *et al.*, 2003; Miura *et al.*, 2003). Diets were prepared every other week and were stored at 4° before use. We did not check the diets for the stability of any of the ingredients; purified diets, however, are generally stable for over one month at refrigerator temperatures [Fullerton, 1982 #10965].

Experimental Design

The experimental protocols and procedures that involved rats were approved by the Institutional Animal Care and Use committee of the University of Kentucky and were in accordance with all policies for the use and care of laboratory research animals as stipulated by the NIH. Eighty female weanling Sprague-Dawley rats weighing 100–125g at arrival (Harlan Sprague Dawley, Indianapolis, IN), were housed three per cage in a temperature- and light-controlled room. Upon arrival, the animals were fed an unrefined diet and allowed to adjust for one week before starting the experiment. All animals received a single dose of DEN (150 mg/kg) by oral intubation. One week after the DEN administration, rats were provided with either a purified diet alone (Table 1) or with one of the antioxidant diets ad libitum for the remainder of the study. Body weights and food consumption were measured twice per week during the experiment.

After being fed the experimental diets for 21 days, 2/3 of the control animals and all antioxidant diet-fed animals were given four i.p. injections of 300 μmol/kg of PCB 77 every 14 days (Figure 1). Ten days after the last PCB injection, all animals were euthanized, by overexposure to carbon dioxide gas. Three days before euthanasia, Alzet osmotic pumps containing BrdU solution (20 mg/ml) were subcutaneously implanted in all animals. Immediately after euthanasia, a piece of liver from each of four lobes was removed from all animals, fixed in buffered neutral formalin, and then made into paraffin blocks. The remainder of the liver was frozen in liquid nitrogen and then stored at −80°C.

Immunohistochemical Staining

The formalin-fixed liver tissues were paraffin-embedded, sectioned, laid on glass slides, and then double-immunostained with an anti-BrdU antibody first and an anti-PGST antibody second, to identify nuclei that had incorporated BrdU and PGST positive preneoplastic foci, respectively, and finally counterstained with hematoxylin. The Vectastain ABC and ABC-AP Kit (Vector Laboratories) were used for this staining according to the protocol provided by the manufacturer.

Quantitation of Altered Hepatic Foci

The number and volume of PGST-positive foci were measured using a computer digitizing software system developed at University of Wisconsin (Campbell *et al.*, 1982; Campbell *et al.*, 1986; Xu *et al.*, 1998). The images were captured using a Nikon Eclipse E800 microscope equipped with MACRO 0.5x and 1.0x lenses. The number of foci/cm^3 (Saltykov method), foci/liver (Saltykov method), the mean focal volume (Saltykov method), and the volume fraction (Delesse method) were analyzed.

Labeling Indexes

The labeling indexes were quantified in both normal hepatocytes and in PGST-positive foci. At least 3000 nuclei from normal hepatocytes were randomly counted per slide (>1000 in each of three lobes) and the labeling indexes were expressed as the percentage of number of labeled nuclei out of the total number of nuclei counted.

Statistical Analyses

The variables body and liver weights, labeling indexes, mean focal volume, and volume fraction, were analyzed by one-way analysis of variance (ANOVA). The comparisons between the treatments and the control, PCB-77 only, were based on the least squares mean effects of the diets followed by the appropriate statistical test procedure. The least square means provide better estimates of the effects when the treatments have unequal number of replicates. The number of foci per liver and number of foci per cubic centimeter data were analyzed by negative binomial regression model with logarithm as the link function. The statistical comparison was made between group receiving DEN only and the antioxidant treatment groups with the group receiving PCB-77 only. The goodness of fit of the model was assessed by Pearson χ^2 value adjusted for overdispersion and the parameters of the model were estimated by the method of maximum likelihood. The Wald's asymptotic procedure was used to determine the p values for significance of the differences between the PCB treatment groups and the control groups. We used the negative binomial regression model for the analysis of data obtained for the number of foci per liver and number of foci per cubic centimeter because of the discrete nature of these measurements (Espandiari *et al.*, 2003). SAS version 9.1 (SAS Institute, Cary, NC) was used for the above analysis. The results were expressed as means ± standard error of the mean (SEM). The results were considered significant at $p<0.05$.

Results

In this study, the effect of antioxidant phytochemicals on the promoting activity of PCB-77 was determined. After initiation with DEN, rats were fed diets containing several antioxidants while receiving injections of PCB-77 during the promotion period. At the end of the study, the body weights of the rats were not affected by either the PCB-77 or antioxidant treatments (Table 2). The rats receiving DEN + PCB-77 had a significantly higher liver weight and liver weight/body weight ratio compared to rats receiving only DEN. The rats receiving CoQ_{10} had a higher liver weight and liver to body weight ratio than rats receiving PCB-77 alone. None of the other antioxidant phytochemicals affected the liver weight.

The number and volume of preneoplastic foci were quantified using placental glutathione S-transferase (PGST) as a marker. The numbers of PGST-positive foci were quantified as foci/cm^3 and as foci/liver. The volume of the PGST-positive foci was expressed as two endpoints: the mean volume of the foci (in mm^3) and the volume fraction (the percentage of the liver that is occupied by foci), which represents the product of focal number and focal volume (Campbell *et al.*, 1982; Campbell *et al.*, 1986; Xu *et al.*, 1998). The rats receiving DEN + PCB-77 with no antioxidants in the diet had a significant increase in the number and volume of PGST-positive foci compared to rats receiving only DEN (Table 3). None of the phytochemicals significantly altered the number of foci per liver, although lycopene slightly decreased (P = 0.09) and ellagic acid slightly increased (P = 0.08) the number of foci per liver. Lycopene significantly decreased and ellagic acid significantly increased the number of foci per cm^3 compared to rats treated with PCB-77 alone; none of the other phytochemicals had a significant effect, although β-carotene slightly decreased the number of foci per cm^3 (P = 0.09). None of the phytochemicals altered the percent of liver volume occupied by foci compared to the DEN +PCB-77 treated rats, although β-carotene slightly decreased the volume fraction (P = 0.10). However, ellagic acid, CoQ10, and curcumin all significantly decreased the mean volume of foci compared to the DEN + PCB-77 treated rats; in addition, lycopene slightly increased the mean focal volume (P = 0.06).

Cell proliferation was quantified by administering BrdU in Alzet osmotic pumps for three days before euthanasia and then quantifying labeling indexes in tissue sections. In PGST-negative hepatocytes, PCB-77 increased BrdU labeling about two-fold when compared to the group receiving DEN but no PCB-77, but this difference was not statistically significant (P = 0.07).

The labeling index was significantly higher in the CoQ10 group compared to DEN+PCB-77 group, but no other phytochemicals produced a significant effect. Labeling indexes were higher in PGST-positive foci than in normal hepatocytes. The rats receiving DEN but no PCB-77 had a lower labeling index in PGST-positive foci than the rats receiving DEN + PCB-77, but there were no significant differences between the PCB-77 only group and the other groups.

Discussion

In this study we have examined the ability of several antioxidant phytochemicals to inhibit the hepatic promoting activity of PCB-77. None of the agents tested produced a clear decrease in both the number and volume of foci induced. Lycopene and β-carotene decreased the number of foci induced, but either did not affect or increased the mean volume of the foci. Ellagic acid, CoQ10, and curcumin all significantly decreased the mean volume of foci, but either did not affect or increased the number of foci induced. A number of other agents have been observed to have opposite effects on focal number and focal size. Stemm et al. (2008) found that dietary selenium increased the number of foci but decreased their size in PCB-treated rats. Kobusch et al. (1989), however, found that PCB-77 increased the size of N-nitrosomorpholine-initiated foci but decreased the number induced.

β-Carotene produced slight decreases in the number of foci induced as well as in the volume fraction. These results are in general agreement with other experimental studies examining β-carotene. Several studies have examined the effect of β-carotene on liver carcinogenesis using p.o. injections or feeding it in the diet or drinking water at concentrations ranging from 0.01–0.1%, and most, but not all, observed a protective effect (Moreno *et al.*, 1991; Murakoshi *et al.*, 1992; Sarkar *et al.*, 1994; Hirose *et al.*, 1995; Astorg *et al.*, 1996; Sadek and Hayat, 1996; Tsuda *et al.*, 1996; Rizzi *et al.*, 1997; Dagli *et al.*, 1998; Gradelet *et al.*, 1998; Bishayee *et al.*, 2000; Moreno *et al.*, 2002; Takasuka *et al.*, 2002; Chattopadhyay *et al.*, 2004; de Almeida Vasconcelos Fonseca *et al.*, 2005). The dietary concentration used in the present study (0.5%) was higher than in the other dietary studies, but did not result in a greater inhibition of carcinogenesis. In experimental carcinogenesis studies in other tissues, most studies have observed a protective effect of β-carotene (Toma *et al.*, 1995; Nishino *et al.*, 2002; Russell, 2004). However, in the human ATBC and CARET clinical trials, β-carotene was found to enhance the development of lung cancer in smokers (Heinonen *et al.*, 1994; Omenn *et al.*, 1996a; Omenn *et al.*, 1996b).

Ellagic acid was found to increase the number of foci but to decrease their mean volume. In other studies examining the liver, ellagic acid (1% in diet) was similarly found to increase the number of PGST-positive foci in a multi-organ carcinogenesis model (Akagi *et al.*, 1995). Ellagic acid (0.04% in diet), however, was found to inhibit 2-acetylaminofluorene (AAF)-induced liver tumors (Tanaka *et al.*, 1988). Therefore the intermediate dose used in the present study (0.4% in diet) is more in agreement with the high-dose study. Ellagic acid additionally has been found to be anti-carcinogenic in other tissues (Wood *et al.*, 1982; Mukhtar *et al.*, 1986; Hannum, 2004).

Curcumin did not affect the number of foci induced, but significantly decreased their mean volume. In previous studies in the liver, curcumin has been found to inhibit the induction of altered hepatic foci by DEN (0.2% curcumin in diet) (Chuang *et al.*, 2000) or by DEN/AAF (200 mg curcumin/kg body weight for 5 days) (Shukla and Arora, 2003). However, curcumin (0.5% in diet) was found to enhance the promotion of altered hepatic foci by 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ) (Hirose *et al.*, 1999). Dietary curcumin (0.5% in diet) did not affect the incidence of spontaneous liver tumors in Long-Evans Cinnamon rats (Frank *et al.*, 2003), which could be related to an elevation in lipid peroxidation-related DNA adducts in curcumin-fed rats (Nair *et al.*, 2005). The dose used in the present study (0.5%) was

similar to that used in these other studies. Curcumin (0.1–0.2% in diet) was found to prevent colon carcinogenesis in several studies (Rao *et al*., 1995; Kawamori *et al*., 1999; Mahmoud *et al.*, 2000). Most other carcinogenesis studies in other tissues have also observed an inhibition in tumor induction after curcumin feeding (Joe *et al.*, 2004; Thangapazham *et al*., 2006).

Lycopene decreased the number of foci induced but slightly increased their volume. Other studies that examined the role of lycopene in hepatocarcinogenesis saw mixed results. Astorg and colleagues found that lycopene (0.03% in diet) decreased the initiating activity of DEN but not that of 2-nitropropane or aflatoxin B1 (Astorg *et al.*, 1997; Gradelet *et al.*, 1998). Lycopene (0.2 mg p.o. 3x/wk) was found to decrease the incidence and multiplicity of spontaneous liver tumors in C3H mice (Nishino, 1997), but did not significantly affect the incidence of spontaneous liver tumors in Long-Evans Cinnamon rats (when fed at 0.005% of diet) (Watanabe *et al*., 2001). Breinholt et al. (2003), however, found that lycopene (0.005% in diet) induced a low level of PGST-positive foci in rats. The use of the Lycovit in the present study makes the present study somewhat difficult to compare with the other studies, but the higher dose used may have been effective in lycopene's ability to decrease the number of foci induced. In other tissues, most studies have observed a chemopreventive effect of lycopene (Bhuvaneswari and Nagini, 2005).

Co-enzyme Q_{10} decreased the mean volume of foci but did not affect the number induced. No other studies have examined the effect of coenzyme Q_{10} on liver carcinogenesis. In studies in other tissues, coenzyme Q_{10} was found to reduce the volume of DMBA-induced mammary tumors (40 mg/kg body wt/day p.o. for 28 days) (Perumal *et al.*, 2005), the number of colon tumors induced by dimethylhydrazine (200 μg/day for 26 wk) (Suzuki *et al.*, 1986), and the number of aberrant crypt foci induced by azoxymethane (0.02% or 0.05% in diet) (Sakano *et al.*, 2006).

Although the tea extract did not affect the promotion of foci by PCB-77 in this study, most other studies have found tea extracts and EGCG to be inhibitory in liver carcinogenesis models. Both green tea (0.63 or 1.25% in drinking water for 40 wk) and black tea (1.25% in drinking water) were found to inhibit the induction of hepatic tumors by DEN in C3H mice, but no association between EGCG content and chemopreventive effect was observed (Cao *et al.*, 1996). Green tea (2% in drinking water) was found to prevent the promotion of hepatic tumors by pentachlorophenol (PCP) in mice (Umemura *et al*., 2003). Green tea catechins (1% in diet) were found to inhibit the promotion of altered hepatic foci by MeIQ or 2-amino-6-methyldipyrido[1,2-a:3′,2′-d]imidazole (Glu-P-1) in rats (Hirose *et al.*, 1995; Hirose *et al*., 1999). In addition, Mao (1993) found that an epicatechin complex inhibited the initiation of hepatocarcinogenesis by DEN. Purified EGCG as well as other purified catechins and tea extracts (0.05 or 0.1% catechin in diet) were found to inhibit the induction of PGST-positive foci by DEN and phenobarbital (Matsumoto *et al.*, 1996). However, green tea catechins (1% in diet) increased the induction of PGST-positive foci using a multi-organ rat carcinogenesis model (Hirose *et al.*, 1993). A green tea extract (0.01 or 0.1% in drinking water) was found to inhibit the number but not the size of DEN-induced tumors in rats but did not affect tumors induced by choline deficiency (Tamura *et al*., 1997). There does not appear to be a correlation between dose and the effect of tea components in the published studies, but comparisons are difficult due to differences in the tea extracts and route of administration (feed vs. drinking water). In other tissues, EGCG as well as tea or tea extracts generally were found to inhibit carcinogenesis (Yang *et al.*, 2002).

Neither NAC nor resveratrol had an effect in this study. In other liver carcinogenesis models, NAC (10–100 mg/kg body weight p.o. 5x/wk) inhibited the induction of PGST-positive foci by 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) in rats (Nishikawa-Ogawa *et al.*, 2006), but did not affect the induction of liver tumors in rats treated with DEN and

diethyldithiocarbamate when administered at 400 mg/kg body weight/day in drinking water (Balansky *et al.*, 2002). Resveratrol, injected at a dose of 1 mg/kg body weight/day i.p. for 7 days (Carbo *et al.*, 1999) or at 5, 10 or 15 mg/kg body weight/day i.p. for 10 days (Wu *et al.*, 2004), was found to decrease the growth of a transplantable hepatoma. It is difficult to compare these results with those from the present study since we fed these agents in the diet whereas the other studies administered them in the drinking water or by injection. In other tissues, resveratrol inhibited chemically-induced carcinogenesis in the skin, mammary gland, and colon, but had no effect in the lung (Yang *et al.*, 2001).

Other dietary antioxidants have been examined for their effect on the promotion of liver carcinogenesis by PCBs. Stemm et al. (2008) found that selenium supplementation increased the number of PGST-positive foci in PCB-77-treated rats, but that it reduced the mean focal volume of the foci in untreated, PCB-77-treated, and PCB-153-treated rats. Glauert et al. (2005) found that dietary vitamin E did not affect the promotion of PGST-positive foci by either PCB-77 or PCB-153.

The only phytochemical that affected cell proliferation was CoQ_{10}, which increased the labeling index in normal hepatocytes (but which did not affect the labeling index in PGST-positive foci). This increase in normal hepatocytes correlated with CoQ_{10}'s effect on the liver weight, which also was increased. It is possible that these two endpoints indicate toxicity from the CoQ_{10} but they could also represent additive hyperplasia. However, body weight was not affected by CoQ_{10} or any of the other phytochemicals. Several of the phytochemicals (CoQ_{10}, curcumin, and ellagic acid) significantly decreased the mean focal volume and one (lycopene) slightly increased it, but none of these phytochemicals affected the labeling index in PGST-positive foci ($P > 0.30$ for all phytochemicals). The rats not receiving PCB-77 had a lower labeling index in both normal hepatocytes and PGST-positive foci, which correlated with the decrease both in the liver weight and in the number and volume of foci induced.

In summary, we observed that some antioxidant phytochemicals can influence the promoting activity of PCB-77. However, none of the agents produced a clear decrease in both the number and volume of PGST-positive foci induced. Therefore the activity of these antioxidant phytochemicals as chemopreventive agents in PCB-induced carcinogenesis would appear to be limited. Only one of the PCBs that have promoting activity in the liver was studied, however, so it is possible that one or more of these phytochemicals could have a major effect on another PCB that has promoting activity. The results of this study, in combination with the studies showing that vitamin E and selenium also do not clearly decrease the promoting activities of PCB-77 and PCB-153 (Glauert *et al.*, 2005; Stemm *et al.*, 2008), imply that dietary antioxidants are not effective at inhibiting tumor promotion by PCBs. These results also indicate that the induction of oxidative stress by PCBs may not be a mechanism in the promoting activities of these agents. Other mechanisms, such as direct effects on signal transduction pathways or effects on vitamin A metabolism, may be more important (Glauert *et al.*, 2001).

Acknowledgements

The authors would like to thank Dr. Zaineb Fadhel, Amy Dugan, Amita Kumar, Jill Cholewa, and Sam Patel for their assistance with the study. This work was supported by grants from the National Institutes of Health (ES07380, ES012475, and ES013661) and by the Kentucky Agricultural Experiment Station.

Abbreviations

AAF

2-acetylaminofluorene

ANOVA

analysis of variance

BrdU
bromodeoxyuridine

CoQ_{10}
co-enzyme CoQ_{10}

DEN
diethylnitrosamine

EGCG
epigallocatechin-3-gallate

GC-MS
gas chromatography-mass spectrometry

Glu-P-1,
2-amino-6-methyldipyrido[1,2-a: 3′,2′-d]imidazole

MeIQ
2-amino-3,4-dimethylimidazo[4,5-f]quinoline

NAC
N-acetyl cysteine

PCB-77
3,3′,4′4-tetrachlorobiphenyl

PCBs
polychlorinated biphenyls

PGST
placental glutathione S-transferase

SEM
standard error of the mean

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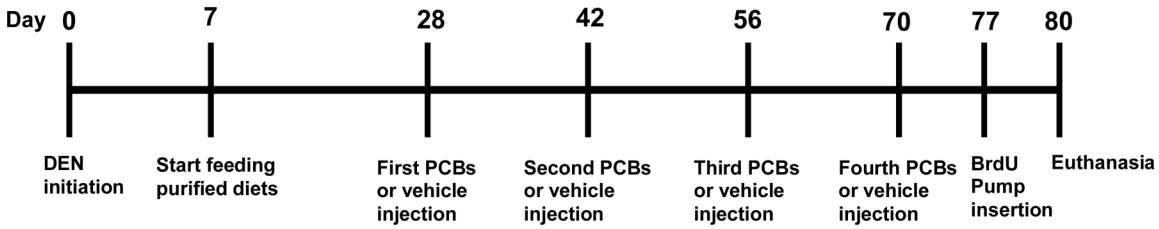

Figure 1.
Experimental Design

Table 1

Composition of Purified Diets

Dietary Ingredient	% of diet
Casein	14.00
Cornstarch	46.57
Dextrose monohydrate	25.5
Soybean oil	4.00
Cellulose fiber	5.00
AIN-93 mineral mix	3.50
AIN-93 vitamin mix	1.00
Choline bitartrate	0.25
L-Cystine	0.18

Table 2

Body and Liver Weights

Treatment	Rats per treatment	Body weight (gm)	Liver weight (gm)	Liver weight/ body weight (%)
No PCB-77				
Control diet	4	266 ± 9	8.8 ± 0.3*	3.3 ± 0.1*
PCB-77 Treated				
Control diet	9	256 ± 2	14.0 ± 0.6	5.4 ± 0.2
β-carotene	8	255 ± 4	14.4 ± 0.3	5.7 ± 0.2
CoQ10	9	256 ± 4	15.2 ± 0.4*	5.9 ± 0.2*
Curcumin	9	255 ± 4	14.0 ± 0.4	5.5 ± 0.2
Tea extract	8	262 ± 4	14.3 ± 0.3	5.5 ± 0.1
Ellagic acid	9	258 ± 5	13.5 ± 0.4	5.2 ± 0.1
Lycopene	6	259 ± 4	14.6 ± 0.2	5.7 ± 0.1
NAC	9	251 ± 3	13.5 ± 0.5	5.4 ± 0.2
Resveratrol	9	260 ± 4	13.9 ± 0.3	5.4 ± 0.1

Values are means ± SEM.

* Significantly different from the PCB-77-treated control diet group ($P \leq 0.05$)

Table 3

Effect of Antioxidant Phytochemicals on the Promotion of PGST-Positive Foci by PCB-77

Treatment	Foci/Liver	Foci/cm^3	Mean Focal Volume (mm^3 × 10^{-3})	Focal Volume (% of Liver)
No PCB-77				
Control diet	3182 ± 842*	361 ± 93*	4.6 ± 0.24*	0.17 ± 0.05*
PCB-77 Treated				
Control diet	29449 ± 3772	2123 ± 278	6.5 ± 0.66	1.3 ± 0.18
β-Carotene	21677 ± 2845	1511 ± 214	5.8 ± 0.45	0.85 ± 0.09
CoQ10	28330 ± 4030	1878 ± 280	4.9 ± 0.71*	1.1 ± 0.21
Curcumin	27108 ± 3896	1930 ± 261	4.9 ± 0.61*	0.9 ± 0.13
Tea extract	25717 ± 6530	1803 ± 453	6.7 ± 0.52	1.2 ± 0.3
Ellagic acid	40940 ± 5425	3065 ± 431*	4.8 ± 0.52*	1.5 ± 0.33
Lycopene	20630 ± 2400	1407 ± 157*	8.0 ± 0.69	1.1 ± 0.2
NAC	31210 ± 3212	2319 ± 240	5.4 ± 0.22	1.2 ± 0.08
Resveratrol	29241 ± 3341	2109 ± 240	5.5 ± 0.37	1.1 ± 0.13

Data are means ± standard errors.

* Significantly different from the PCB-77-treated control diet group ($P \leq 0.05$)

Table 4

Effect of Antioxidant Phytochemicals on Cell Proliferation in Normal and PGST-Positive Hepatocytes

Treatment	Labeling index: non-focal hepatocytes (%)	Labeling index: PGST-positive hepatocytes (%)
No PCB-77		
Control diet	2.58 ± 1.14	10.0 ± 2.1*
PCB-77 Treated		
Control diet	5.84 ± 0.46	17.8 ± 1.4
β-carotene	5.43 ± 0.69	15.0 ± 1.3
CoQ10	13.06 ± 1.88*	20.1 ± 1.7
Curcumin	5.51 ± 0.85	18.8 ± 2.6
Tea extract	6.68 ± 0.96	20.1 ± 2.6
Ellagic acid	5.25 ± 0.47	14.7 ± 1.7
Lycopene	4.85 ± 0.30	15.7 ± 1.5
NAC	5.21 ± 0.44	20.1 ± 2.5
Resveratrol	6.81 ± 1.41	21.5 ± 3.4

Data are means ± standard errors.

*Significantly different from the PCB-77-treated control diet group ($P \leq 0.05$)