Activation of the Transcription Factors NF-κB and AP-1 during the Promotion of Hepatocarcinogenesis by Polychlorinated Biphenyls (PCBs)

Howard P. Glauert, Job C. Tharappel, Eun Y. Lee*,
Larry W. Robertson***, and Brett T. Spear*·**

Departments of Nutrition and Food Science, *Pathology and Laboratory Medicine,and **Microbiology and Immunology; and ***Graduate Center for Toxicology; University of Kentucky; Lexington, KY 40506

Introduction

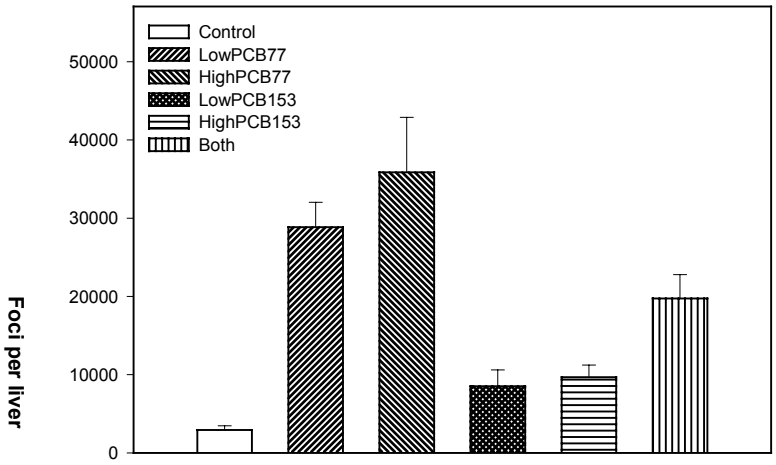
It is well established that polychlorinated biphenyls (PCBs) are efficacious inducers of hepatocellular carcinomas in rodents (1). Both PCB mixtures and individual PCB congeners have promoting activity in two-stage hepatocarcinogenesis, but the mechanism of their promoting activity is unclear. A number of mechanisms have been proposed, including induction of toxicity, induction of oxidative damage, and effects on vitamin A metabolism (1). One mechanism by which PCBs may promote hepatic tumors is by inducing oxidative damage in the liver. Several but not all studies have found that PCBs increase hepatic lipid peroxidation (1). Certain congeneric PCBs administered for short time periods were found to increase the levels of oxidative DNA damage, using 8-hydroxyguanosine as the endpoint (2).

Another mechanism by which oxidative stress from tumor promoters can influence carcinogenesis is by altering gene expression in the cell. For example, the transcription factors NF-κB and AP-1 have been shown to be activated by oxidative stress (3-6). Recently we observed that several hepatic tumor promoting agents (phenobarbital and the peroxisome proliferators) increased the DNA binding activity of the transcription factor NF-κB in the liver (7-9). Ciprofibrate was also found to activate the transcription factor AP-1 (9). In this study, we examined whether the administration of PCBs would increase the hepatic activation of the transcription factors NF-κB and AP-1.

Materials and Methods

Female Sprague Dawley rats were obtained from Harlan Sprague Dawley (Indianapolis, IN). All the animals in this study received a single dose of diethylnitrosamine (DEN, 150 mg/kg). After a two-week recovery period, rats received four biweekly injections of 3,3',4,4'-tetrachlorobiphenyl (PCB-77) or 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153) (100 or 300 μmol/kg), or both PCBs (100 μmol/kg each). 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 (10). The rate of hepatic DNA synthesis was estimated by quantifying labeling indexes of BrdU-stained nuclei.

Figure 1. Effect of PCBs on the Number of PGST-positive Foci per Liver.

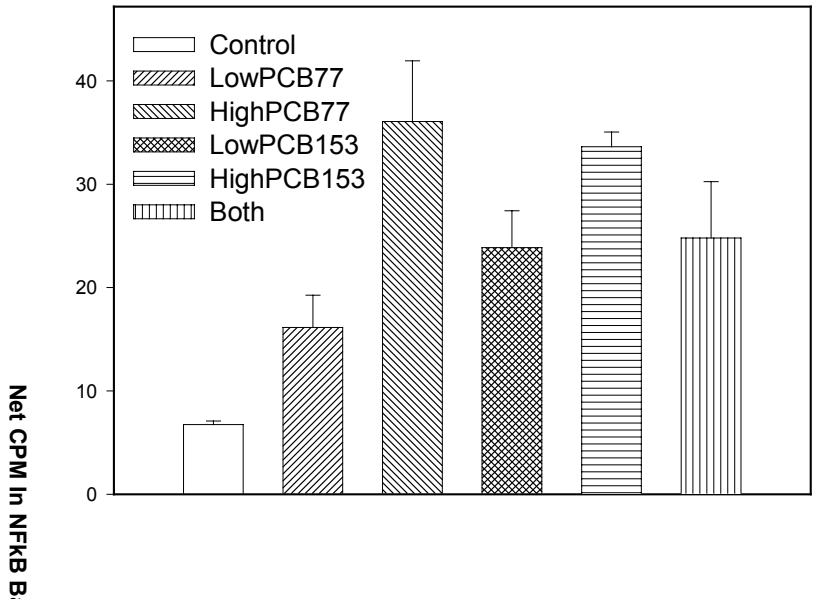


The DNA binding activities of NF-κB and AP-1 were determined using electrophoretic mobility shift assays (EMSAs). Nuclear extracts were prepared from the frozen liver samples (11). The DNA binding activity for each transcription factor was determined in these nuclear extracts using EMSAs, as we have described previously (9). Samples were run on a 6% polyacrylamide gel and then autoradiographed. Densitometry or phosphoimaging was used to quantify the amount of radioactivity in each band.

Results and Discussion

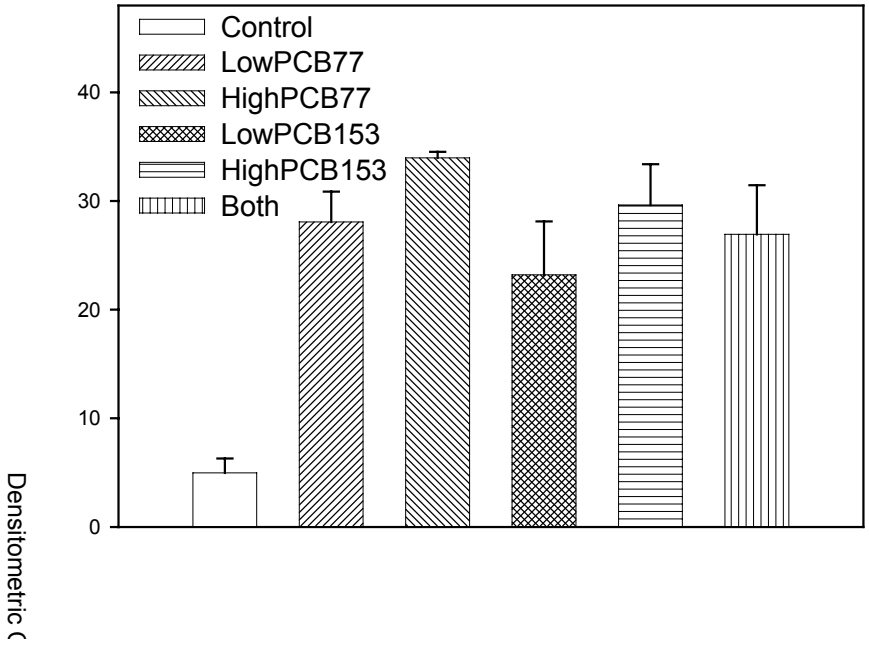
The number of PGST-positive foci per liver were increased in rats administered PCBs, with the highest increase seen in rats administered PCB-77 (Figure 1). The number of PGST-positive foci in rats receiving both PCBs was intermediate between those receiving the low doses of either PCB-77 or PCB-153, demonstrating that a synergistic effect does not exist between these two PCBs. The mean volume of foci, however, was only increased 20-40% in PCB-treated rats (data not shown), which was not statistically significant. Therefore the main effect of PCBs on tumor promotion in this model was to increase the number of PGST-positive foci rather than their volume. The BrdU labeling index was measured in both focal and non-focal tissue in the liver. In non-focal hepatocytes, the labeling index was significantly increased only in the rats receiving the high dose of PCB-77. In focal hepatocytes, only the highest dose of PCB-77 significantly increased the labeling index. There was a significant increase in NF-κB and AP-1 binding activities in hepatic nuclear extracts from rats in the high dose groups and in rats receiving both PCBs (Figures 2 and 3).

Figure 2. Effect of PCBs on the Activation of NF-κB



The mechanisms of the activations of NF-κB and AP-1 by PCBs are not known. Active oxygen in the form of superoxide or hydrogen peroxide can be released as a by-product from cytochrome P-450 (12) and thus could contribute to NF-κB and AP-1 activation. Another mechanism by which coplanar PCBs could influence NF-κB activation is through their activation of the *Ah* receptor. The activated *Ah* receptor has recently been found to bind to the p65 subunit of NF-κB (13). However, our finding that the DNA binding activity of NF-κB was strongly induced after 4 injections of PCB-77 implies that the cell is able to overcome this negative effect on NF-κB activation.

Figure 3. Effect of PCBs on the Activation of AP-1



Acknowledgments

This research was funded by National Institute for Environmental Health Sciences grants ES07380 and ES07266, and by the Kentucky Agricultural Experimental Station.

References

1. Silberhorn EM, Glauert HP, and Robertson LW; *Crit. Rev. Toxicol.* **1990**, 20, 439-496.
2. Twaroski TP, Arif JM, Gupta RC, and Robertson LW; *Proc. Am. Assoc. Cancer Res.* **1999**, 40, 647-648.
3. Meyer M, Schreck R, and Baeuerle PA; *EMBO J.* **1993**, 12, 2005-2015.
4. Liu SL, Esposti SD, Yao T, Diehl AM, and Zern MA; *Hepatology* **1995**, 22, 1474-1481.
5. Amstad PA, Krupitza G, and Cerutti PA; *Cancer Res.* **1992**, 52, 3952-3960.
6. Abate C, Patel L, Rauscher F, and Curran T; *Science* **1990**, 248, 189-194.
7. Li Y, Leung LK, Glauert HP, and Spear BT; *Carcinogenesis* **1996**, 17, 2305-2309.
8. Li Y, Leung LK, Spear BT, and Glauert HP; *Biochem. Biophys. Res. Commun.* **1996**, 229, 982-989.
9. Nilakantan V, Spear BT, and Glauert HP; *Carcinogenesis* **1998**, 19, 631-637.
10. Glauert HP, and Clark TD; *Cancer Lett.* **1989**, 43, 95-100.
11. Deryckere F, and Gannon F; *Biotechniques* **1994**, 16, 405.
12. Bondy SC, and Naderi S; *Biochem. Pharmacol.* **1994**, 48, 155-159.
13. Tian Y, Ke S, Denison MS, Rabson AB, and Gallo MA; *J. Biol. Chem.* **1999**, 274, 510-515.