Role of NF-κB in the promotion of hepatocarcinogenesis by chemicals

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The purpose of these studies was to determine if hepatic tumor promoters could activate the transcription factor NF-κB, the mechanism of this activation, and whether activation of NF-κB is important in the carcinogenic process. We first demonstrated that the administration of the peroxisome proliferator ciprofibrate increases the hepatic DNA binding activity of NF-κB in rats and mice. Peroxisome proliferators induce the peroxisomal β-oxidation pathway, which produces hydrogen peroxide as a by-product. We therefore hypothesized that increasing hydrogen peroxide production would increase and increasing cellular antioxidants would block NF-κB activation. To examine if hydrogen peroxide could directly activate NF-κB, we overexpressed the peroxisomal hydrogen peroxide-producing enzyme fatty acyl CoA oxidase (FAO) in Cos cells. When an NF-κB-regulated luciferase reporter gene was co-transfected along with a substrate for FAO (linoleic acid), increased luciferase activity was observed. To examine the effects of antioxidants, we first added vitamin E and N-acetyl cysteine to H4IIEC3 cells, and found them to inhibit peroxisome proliferator-mediated NF-κB activation. Vitamin E also inhibited hepatic NF-κB activation *in vivo*. We also used a transgenic mouse model in which the hydrogen peroxide-metabolizing enzyme catalase was overexpressed specifically in the liver. Catalase overexpression inhibited the DNA binding activity of NF-κB after 21 days of ciprofibrate administration. In addition, the ciprofibrate-induced increase in hepatocyte proliferation was decreased by catalase overexpression, indicating a possible role for NF-κB in cell proliferation by peroxisome proliferators. Finally, NF-κB is not activated by peroxisome proliferators in hamsters, which have much higher levels of the antioxidant enzymes glutathione peroxidase, glutathione S-transferase, and DT-diaphorase. Hamsters are also not responsive to the carcinogenic and cell proliferation-inducing effects of the peroxisome proliferator Wy-14,643. Overall, these results show that hydrogen peroxide production after the administration of ciprofibrate is responsible at least in part for the activation of NF-κB in the liver. Furthermore, these results imply that NF-κB activation is likely important in the induction of cell proliferation by peroxisome proliferators.

NF-κB is also activated by other tumor promoters in the liver. The administration of phenobarbital in the diet increases the hepatic DNA binding activity of NF-κB. Administering vitamin E in the diet to rats inhibited NF-κB activation by phenobarbital, implying that oxidative stress is also involved in NF-κB activation by this tumor promoter. The administration of the PCBs 3,3',4,4'-tetrachlorobiphenyl (PCB-77) or 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153) also increases the hepatic DNA binding activity of NF-κB.

Although the above studies show a correlation between xenobiotic administration and hepatic NF-κB activation, they do not demonstrate a cause and effect relationship between administration of the agent, NF-κB activation, and tumorigenesis. One method for examining whether NF-κB is essential in the carcinogenic or tumor-promoting effects of hepatocarcinogens is to use animal models that are deficient in NF-κB activation. A knockout model has been developed that is deficient in the p50 subunit of NF-κB. We have analyzed the effects of ciprofibrate in these mice. These p50 knockout mice (p50 -/-) and wild type mice were fed either

a control diet or diet containing 0.01% ciprofibrate for 10 days. As measured by electrophoretic mobility shift assays, NF-κB DNA binding activity was present in untreated wild type mice and was increased after ciprofibrate treatment. NF-κB DNA binding activity could not be detected in p50 -/- mice fed the control diet or ciprofibrate. Cell proliferation was measured in these animals by 5-bromo-2'-deoxyuridine (BrdU) labeling. The untreated p50 -/- mice had a higher BrdU labeling index than did untreated wild type mice. However, the increase in proliferation was greater in ciprofibrate-fed wild type mice than in ciprofibrate-fed p50 -/- mice. These data indicate that NF-κB may be involved in some of the proliferative changes that occur in the liver in response to ciprofibrate.