Expression of a Dioxin-Specific Gene in the Liver of Sprague-Dawley Rats

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Chronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) caused an increased incidence of hepatocellular carcinomas in the female Sprague-Dawley rat[1]. Recently 4 genes have been cloned from a human keratinocyte cell line that exhibit dioxin-induced transcriptional activation[2]. Two of these genes have been identified as plasminogen activator inhibitor-2 (PAI-2) and interleukin-1β (IL1-β). The two remaining clones (1 and 141) have not been identified. The role of these 4 genes in dioxin-induced toxicity and/or carcinogenicity in rats is unknown. The experimental question addressed in the present study is whether these dioxin responsive genes from human skin are expressed in the liver of Sprague-Dawley rats. We also examined the dose response relationship of dioxin-induced gene expression and determined whether expression was linked to hepatic cell proliferation.

^{3}H-TCDD was administered using a dose loading/maintenance regimen to achieve a rapid steady-state dioxin concentration within the liver of 0.12, 30 and 150 ng/gm of tissue. The 30 ng/gm dioxin concentration, the final liver concentration observed in the high dose of the cancer bioassay, resulted in an increased tumor incidence. Loading and maintenance doses required to achieve the desired steady-state tissue concentrations were determined prospectively using a PB/PK model for dioxin developed by Andersen et al.[3]. Direct determination of dioxin concentration in the liver of exposed animals verified that targeted concentrations were achieved.

Because the 4 dioxin responsive genes were cloned from human skin, Southern hybridization analysis was conducted with rat liver DNA to determine if the human probes recognize the homologous rat genes. Under the conditions of this analysis, only clone 1 hybridized to the rat DNA. Consequently, our analysis to determine whether dioxin could induce altered transcription in the rat liver was initially limited to clone 1.

Gene expression was determined by Northern analysis using poly A mRNA isolated from liver tissue from animals exposed to dioxin for 1 and 14 days. As expected, dioxin induced the expression of the cyp1a1 gene in a dose-dependent manner. There was a dose related expression of clone 1 at both day 1 and 14 in the liver of female animals exposed to dioxin at the intermediate and high doses. Northern analysis was also conducted to evaluate whether the expression of clone 1 was associated with hepatic cell proliferation or by exposure to hepatic tumor promoting agents. Using poly A mRNA isolated from animals

subjected to either a 2/3 partial hepatectomy or exposure to a single administration of the peroxisome proliferator Wy-14,643, carbon tetrachloride or phenobarbital, at doses of these chemicals that induced hepatic cell proliferation, clone 1 was not detected under any of these experimental conditions.

Liver weights in rats exposed to the mid- and high-doses of dioxin were elevated compared to control animals. No elevation of liver-specific serum enzymes was noted in treated animals, nor was there any histological evidence for dioxin-induced liver necrosis, indicating a lack of overt hepatotoxicity under these experimental conditions. Hepatocyte proliferation was determined immunohistochemically after 5-bromo-2'-deoxyuridine administration via osmotic pumps implanted 6 days before the animals were sacrificed. An increase in cell proliferation was not observed at either 7 or 14 days of dioxin treatment.

In conclusion, exposure to dioxin for either 1 or 14 days resulted in the dose response expression of the transcriptionally induced human clone 1 gene in the liver of female Sprague-Dawley rats. Slight increases in hepatocyte proliferation have been reported after chronic dioxin exposure[4]. Under the current treatment regimen dioxin did not cause an increase in hepatic cell proliferation after either 1 or 2 weeks of treatment. Expression of clone 1 does not appear to be linked to cell proliferation, as hepatic expression was observed in its absence and not observed under conditions of increased hepatic cell proliferation. Studies into the mechanisms of gene expression by dioxin in the rat liver should increase the understanding of how dioxin leads to the development of tumors in this organ.

References

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