Mechanisms of dioxin-induced hepatocarcinogenesis in rats.

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Introduction

While 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a multi-site carcinogen, the induction of liver tumors by TCDD in the female Sprague–Dawley rat has been consistently used by regulatory agencies in the establishment of appropriate guidelines for human exposure to dioxins. However, the mechanism of tumor induction by TCDD in the rat liver is not known. This knowledge gap has often been used to foster the debate that the conservation of mechanism of action of transcriptional activation via the aromatic hydrocarbon receptor (AHR) between rodents and humans may not necessarily predict a conserved mechanism of carcinogenicity or potential carcinogenic risk. An understanding of the mechanism of liver tumor promotion by TCDD would provide valuable information in evaluating the carcinogenic risk posed by human exposure to dioxin-like compounds.

Materials and Methods

Our studies on the mechanism of carcinogenesis induced by TCDD have focussed on the female Sprague-Dawley rat, and in particular on the induction of liver tumors by TCDD within the framework of a chronic two-stage initiation-promotion protocol. Animals, initiated with either DEN or vehicle control, were treated with TCDD using a daily averaged dose of 125 ng/kg/day for up to 60 weeks. For the evaluation of liver tumor promotion, we measured the development of putatively preneoplastic foci of enzyme-altered hepatocytes that exhibit expression of the marker enzymes placental glutathione–s-transferase (PGST) and gamma-glutamyl transpeptidase (GGT). The development of these lesions is believed to be a part of the sequence of events leading to liver tumor development. The quantitation of the number of these lesions in the liver, the mean size of the lesions and the amount of liver occupied by them, can be used to evaluate the action of liver tumor promoters. Alteration in hepatocyte proliferation was measured by immunohistochemical analysis of the incorporation of bromodeoxyuridine into replicating hepatocytes *in vivo*.

Results and Discussion

Time course and reversibility of liver tumor promotion

In a chronic two-stage initiation/promotion studies, significant increases in non focal hepatocyte cell proliferation and enzyme-altered hepatic foci formation are observed in the livers of female Sprague-Dawley rats treated biweekly with a daily dose of 125 ng TCDD/kg/day for 30 weeks[1]. Time course analysis indicated that continuous dosing with TCDD induced an exposure duration dependent induction of liver tumor promotion as assessed by staining for altered hepatic foci expressing the placental form of glutathione s-transferase (PGST)[2]. PGST positive foci are believed to be preneoplastic lesions and therefore the analysis of the development of these lesions

is used in studies of the mechanism tumor promotion. The number per unit area, the percent of liver occupied by foci (liver focal burden) and the mean size of PGST foci were all increased by TCDD relative to that observed in animals receiving corn oil alone[2].

The promotion of preneoplastic lesions requires continuous exposure to TCDD. After cessation of TCDD treatment there is a time dependent decrease in both the number of lesions per unit volume and also the percentage of the liver occupied by these lesions[2]. Cessation of treatment with TCDD results in a decrease in the amount of TCDD and subsequent decrease in the induced level of CYP1A1 in these livers. Similarly the TCDD-induced increases in non-focal hepatocyte replication is also reversible[3].

The reversibility of preneoplastic lesion development is also reflected in a reduction in the incidence of liver tumors at a later time point relative to animals that were continuously treated with TCDD. Furthermore the incidence of liver tumors in animals treated with TCDD for 30 weeks followed by 30 weeks of corn oil is lower than in animals treated with just corn oil alone[2]. All these data taken together indicate that tumor promotion by TCDD is reversible and that in addition to the magnitude of exposure, the duration of exposure to TCDD is an important factor in determining tumor risk in association with TCDD.

Role of TCDD-induced cell proliferation

It was observed that after either 15 weeks of TCDD exposure (125 ng TCDD/kg/day), a time at which the liver burden of TCDD has reached >95% steady state, that there was no significant increase in non-focal hepatocyte cell replication, as measured by the number of non-focal hepatocyte nuclei incorporating bromodeoxyuridine (BrdU) *in vivo* [3]. Indeed there is a significant reduction in the rate of cell proliferation after TCDD exposure. These data suggest that mito-inhibition of normal hepatocytes at early time points may be a contributing factor in the establishment of an environment that affords a selective growth advantage to focal cells. In contrast after 30 weeks of exposure there was a significant increase in cell proliferation. These data indicate the alteration in cell proliferation by TCDD is exposure duration dependent and not directly related to the level of dioxin in the target organ at a specific time. Recent data indicates that there is no increase but a significant decrease in the BrdU LI in animals exposed to TCDD for 20 weeks. Therefore there appears to be a critical window between at least 20 and 30 weeks of TCDD exposure where significant changes occur in the hepatocyte response to chronic TCDD exposure.

Role of Ovarian hormones in liver tumor promotion

Several lines of evidence from chronic animal studies suggest that ovarian hormones are involved in the mechanism of tumor promotion by TCDD; TCDD is a tumor promoter in female but not male rats[4], ovariectomy is inhibitory to the promotion of preneoplastic foci and liver tumors by TCDD[5], the TCDD induction of cell proliferation is not observed in ovariectomized female rats, higher levels of oxidative DNA damage in TCDD treated intact rats compared with TCDD-treated ovariectomized rats[6], TCDD-induced down regulation of the EGFR is not observed in ovariectomized rats[7].

Its is hypothesized that estradiol may be contributing to the mechanism through the activation of estrogen dependent and TCDD-responsive signal transduction pathways and through the formation of potentially genotoxic catechol estrogen metabolites by TCDD-inducible cytochromes P450. A chronic tumor promotion study was conducted to test the hypothesis that supplement estradiol can

compensate for the inhibitory effect of ovariectomy on tumor by TCDD [8]. This involved the treatment of ovariectomized rats with both TCDD and/or implanted 90-day estradiol pellets, within the framework of a chronic two-stage initiation-promotion study. The efficacy of estradiol supplementation was confirmed by the observation of increased serum estradiol levels and increased uterine wet weight [8]. In contrast to a prior study, there was no effect of ovariectomy or estradiol supplementation on the promotion of preneoplastic lesions by TCDD[9]. Indeed, as was observed previously, TCDD induced promotion of putatively preneoplastic foci in both intact and ovariectomized animals. These data suggest that while TCDD-induced carcinogenesis in the female rat liver may be sensitive to the animals hormonal status that there are clearly there are factors other than ovarian hormones that are likely to have a significant impact on tumor promotion. As was observed previously, 30 weeks of TCDD treatment induced non-focal hepatocyte cell proliferation in intact but not ovariectomized rats. However there was no effect of TCDD on cell proliferation in ovariectomized rats treated with both TCDD and estradiol. The observation of TCDD-induced tumor promotion in the absence of increased non-focal cell proliferation suggest that the induction of cell proliferation is likely a homeostatic response to increased focal promotion and that this response is ovarian hormone sensitive but not estrogen-dependent.

The incorporation of tumor promotion and dose-response data into mathematical two-stage models of carcinogenesis suggest that TCDD is both a promoter (alters the net birth rate/death rate of initiated cells) and an activator or carcinogenesis (alters the rate of transition the “normal” to the “initiated” stage)[10]. While the specific mechanisms for “promotion” and “activation” are still unknown, these conclusions are consistent with known alterations by TCDD of growth factor signal transduction pathways, suppression of apoptosis by TCDD [11] and induction of oxidative DNA damage [6].

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