MECHANISTIC ASPECTS OF TCDD-INDUCED CARCINOGENESIS AND IMPLICATIONS FOR RISK-ASSESSMENT

A.M. Tritscher, G.C. Clark, C.H. Sewall, and G.W. Lucier
National Institute of Environmental Health Siences (NIEHS), Research Triangle Park, PO Box 12233, North Carolina 27709, USA

INTRODUCTION AND METHODS

2,3,7,8-TCDD is the most potent and widely studied member of a group of polychlorinated dibenzodioxins, dibenzofurans and polychlorinated biphenyls which exhibit a broad spectrum of biochemical and toxic effects in animals and humans (1). These so called dioxins seem to act through a common mechanism which is not completely understood but requires at least an initial interaction with an intracellular binding protein, designated the Ah-receptor (2).

Dioxins are ubiquitous and persistent environmental contaminants and because of their high toxicity and potent carcinogenicity in animals they are of great public health concern. TCDD is a potent multisite carcinogen in rodents and the liver tumor incidence in female rats is most widely used for current risk assessment (3). One important issue to improve risk-assessment models is the magnitude of response after chronic low dose exposure, especially in relation to the actual tissue concentration rather than exposure.

Previous studies in our laboratory investigated dose-response relationships of TCDD induced Ah-receptor mediated effects in an initiation-promotion model for hepatocarcinogenesis in female Sprague-Dawley rats. These studies demonstrate that after chronic TCDD exposure various responses exhibit different dose-response relationships indicating that the shape of the dose-response curve can not be predicted solely on the basis that the effect is receptor mediated (4,5,6).

The current report tries to further characterize TCDD mediated changes in the same two-stage model of hepatocarcinogenesis with focus on the development of these changes upon withdrawal of TCDD. Female Sprague-Dawley rats were initiated with a single dose of diethylnitrosamine (DEN) at 175mg/kg and subsequently

promoted by biweekly oral gavage of TCDD in corn oil at a dose equivalent to 125ng/kg/day. Animals were sacrificed after 30 weeks of TCDD treatment with and without a following recovery period of 32 weeks. Changes in livers were quantified and compared to changes in livers of rats after 30 weeks and 60 weeks continous TCDD treatment.

In another attempt to provide data to improve risk-assessment, we quantified TCDD concentrations in lungs of female Sprague-Dawley rats after chronic low dose exposure in comparison to the liver, since recent epidemiological studies suggest the lung as target for carcinogenic action of dioxins in humans (7,8).

RESULTS

Lung and Liver TCDD concentration:

After chronic exposure (30 weeks) the tissue concentration in the liver is proportional to the orally administered dose over a wide dose range. In contrast, the concentration in the lung seems to be proportional at very low doses (0.1, 0.3, 1 ng/kg/day) with approximately a 10-fold lower concentration than in the liver. Lung concentration saturates at higher doses. Figure 1 shows the dose-response curves for tissue concentration in liver and lung. It is important to note, that both tissues have detectable TCDD background levels originating from feed intake and possibly the corn oil vehicle (9).

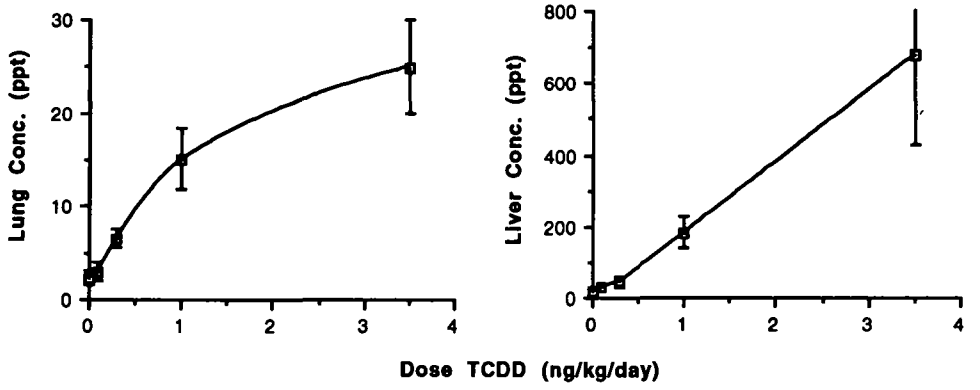

Figure1: TCDD concentrations in lung and liver as a function of the orally administered dose after 30 weeks of chronic treatment within the framework of a two-stage model.

Reversibilty study:

TCDD induced changes in rat liver were compared after 30 weeks chronic exposure with and without a 32 week recovery period. Biochemical changes like induction of cytochrome P4501A1 and 1A2 and changes in EGF receptor decreased in rats with recovery according to decreasing TCDD tissue levels. In contrast, more complex responses as increased cell proliferation and development of putative preneoplastic lesions was still increased. Cell proliferation in non-focal tissue in rats 32 weeks after cessation of TCDD administration was still appr. 3-fold increased over age matched controls as compared to a 4-fold increase over controls after 30 weeks continous exposure. The TCDD liver concentration was approximately 300-times lower at the end of recovery than at the end of 30 weeks dosing. After the recovery the total number of preneoplastic foci with the placental form of gluthathion-S-transferase (PGST) as marker was decreased as compared to animals without recovery but the percent of liver occupied by foci was significantly increased, indicating selective growth of some PGST positive foci even with decreasing TCDD tissue levels. Analysis of serum enzymes showed no indication of liver injury at the end of recovery but the amount of total cholesterol and total bile acids are significantly increased as compared to age matched controls whereas the amount of triglycerides is reduced.

The most surprising finding is that the liver tumor incidence (57%, n=7) after 30 weeks of TCDD exposure followed by 32 weeks of recovery is comperable to the tumor incidence after 60 weeks of continuous TCDD exposure (35%, n=37) (10) but the predominant tumor type is different. After 60 weeks continuous TCDD exposure rats mainly develop hepatocellular adenomas and carcinomas whereas after 30 weeks exposure followed by 32 weeks without further dosing the animals mainly develop bile duct tumors (cholangiomas and cholangiosarcomas). Also, rats treated with TCDD followed by recovery did not develop mammary tumors (7 animals) but 4 out of 11 (36% incidence) age matched control animals developed mammary tumors.

To further investigate these findings we are currently studying changes in rat liver after different periods of TCDD exposure with and without following recovery. In our studies we also include the pharmacokinetics of TCDD concentrations in lungs and liver.

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