TCDD-Induced Lesions In Rat Lung After Chronic Oral Exposure

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Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most potent congener of a group of compounds acting through a specific intracellular binding protein, the aromatic hydrocarbon receptor (AhR)[1]. TCDD is a potent multi-site, multi-species carcinogen in experimental animals of both sexes [2,3]. Several epidemiologic studies suggest that TCDD increases the overall cancer incidence/mortality in occupationally or accidentally highly exposed cohorts and the respiratory tract was identified as one of the potential targets with increased cancer incidence/mortality. These data, together with the conservation of action of TCDD through the AhR support the classification of TCDD as a known human carcinogen [4]. Although the lung appears to be a target organ for TCDD-induced adverse effects in humans there are only few studies in experimental animals which attempt to characterize such effects [5-7]. Therefore we investigated TCDD-mediated effects and their potential reversibility in the lungs of female Sprague-Dawley rats after chronic oral exposure. Within the framework of an initiation-promotion model with diethylnitrosamine (DEN) as initiator and TCDD as promoter we studied systemic effects in lungs after various times of oral TCDD exposure followed by periods without further TCDD administration (withdrawal period). The goal of this time course and reversibility study was to characterize TCDD induces histological changes in the lungs after chronic oral exposure in order to identify target cell populations for future mechanistic studies.

Materials and Methods

Animals, treatment and experimental design

Female Sprague-Dawley rats were randomly divided into 21 groups (6-15 animals per group). At 70 days of age the animals were initiated with a single dose of DEN i.p. at 175 mg/kg in saline vehicle (1 ml/kg of body weight). Two weeks or 18 weeks after initiation, promotion was started with biweekly oral gavage of TCDD in corn oil at a dose of 1750 ng/kg (equivalent to a daily averaged dose of 125ng TCDD/kg/day). All time points included control groups treated with the vehicles only, saline and corn oil, as well as groups initiated only or promoted only. The study design consisted of four basic study arms [8]:

(I) Continuous groups: two weeks after initiation continuous TCDD promotion for 14 (7 x TCDD), 30 (15 x TCDD) or 60 (31 x TCDD) weeks.

(II) Withdrawal groups: two weeks after initiation TCDD promotion for 30 weeks (15 x TCDD) followed by withdrawal period of 16 or 30 weeks.

(III) Waiting groups: 18 weeks after initiation TCDD promotion for 14 (7 x TCDD) or 30 (15 x TCDD) weeks.

(IV) Waiting and withdrawal group: 18 weeks after initiation TCDD promotion for 30 weeks (15 x TCDD) followed by a withdrawal period of 16 weeks.
One week after the last TCDD treatment or one week after the indicated withdrawal periods blood was collected from all animals via cardiac puncture and serum frozen in aliquots in liquid nitrogen until TCDD analysis. The lungs were removed, the left lobe tied off, separated, and frozen in liquid nitrogen for TCDD analysis. The right lung was inflated with 10% neutral buffered formalin (NBF) through the trachea and fixed in 10% NBF overnight.

Histopathology and Immunohistochemistry
Representative sections of the right apical and right diaphragmatic lobe of the lung were dehydrated and embedded in paraffin within 72h of collection. Histopathological evaluation was done on hematoxylin and eosin stained 6 μm sections using standard diagnostic criteria. Immunolocalization of Ah-receptor and CYP1A1 was performed on 5μm sections using a standard avidin-biotin antiperoxidase method as previously detailed[9]. Ah-receptor protein was detected with a rabbit polyclonal antibody, kindly provided by Dr. George Clark, CYP1A1 protein was detected using a mouse monoclonal antibody (PM10, Oxford Biomedical Research, Inc., Oxford, MI).

TCDD Analysis:
TCDD concentrations in lung and serum were quantified by GC-MS as previously described [10]. In the groups including withdrawal periods with low TCDD concentrations, lung lobes and serum samples (1ml aliquots) from two animals were pooled for analysis.

Results and Discussion
Few tumors were observed at the latest time point of the study in animals initiated with DEN and TCDD promoted for 60 weeks or for 30 weeks with following withdrawal period. Observed were alveolar-bronchiolar (AB) tumors in 3 animals. AB adenomas occurred in 2 rats, one promoted with TCDD for 60 weeks (T 60) and the other promoted for 30 weeks and following 30 weeks withdrawal (T 30-30).

Table I summarizes the incidence and severity of all observed lung lesions (except tumors). DEN initiation was associated with *alveolar hyperplasia*, a proliferative change of the alveolar parenchyma in which there is a focal increase in the number of type II epithelial cells lining the interalveolar septae. Airway macrophages were usually present in the hyperplastic foci. Severity of this effect ranged from minimal (single foci, alveolar architecture preserved) to marked (multiple foci or foci with filling and distortion of alveolar spaces). DEN alone was associated with alveolar hyperplasia, detected at the earliest time point in 2 of 6 (33%) animals 33 weeks after injection, and increased in incidence with time to an incidence of 8 of 11 (73%) by 63 weeks after initiation. TCDD treatment did not affect the incidence but did enhance the severity of this lesion. Only a single incidence of this lesion occurred in all treatment groups not initiated with DEN.

TCDD treatment was associated with metaplastic change of the alveolar-bronchiolar (AB) region.*AB metaplasia* was characterized by focal extrabronchiolar proliferation of cuboidal to columnar mucociliary epithelium which extended along alveolar walls and alveolar ducts adjacent to terminal bronchioles. Luminal mucous and foamy macrophages were typically present within these foci as detected by Alcian blue stain. AB metaplasia is a rare lesion, it is generally not considered preneoplastic. The highest incidence and severity of AB metaplasia was observed (6 of 8 animals = 75%) in rats treated continuously with TCDD for 60 weeks without DEN initiation. Continuous TCDD treatment for 30 weeks was not sufficient to induce AB metaplasia, however

this lesion was detected in the groups with 16 weeks of withdrawal after 30 weeks of TCDD treatment, indicating that the development of these lesions is time and TCDD-dependent. After 31 weeks of withdrawal no AB metaplasia was detected, indicating the reversibility of this proliferative lesion. Only 1/11 animals treated with DEN alone exhibited AB metaplasia. This lesion was clearly induced by TCDD alone.

Bronchiolar hyperplasia was seen primarily in DEN-initiated rats and promoted with TCDD for 60 weeks. Bronchiolar hyperplasia was an intrabronchiolar proliferation characterized by focal thickenings of the mucosal lining of small bronchioles due to proliferation of epithelial cells. The highest incidence of bronchiolar hyperplasia occurred in 6 of 14 (43%) rats in the DEN-initiated group promoted for 60 continuous weeks with TCDD. While it is unclear whether this lesion is considered to be preneoplastic the incidence of this lesions was clearly promoted by TCDD treatment

AhR could only be detected in bronchiolar cells, in Clara cells and ciliated cells, and not in alveolar epithelium. CYP1A1 could also only be detected in bronchiolar epithelium, mainly in Clara cells. Localization of a functional AhR in the same cells where proliferative changes were induced by TCDD might indicate a receptor-mediated event. Localization and intensity of staining for AhR were not affected by either DEN or TCDD treatment.

Mean TCDD concentrations in the lung were 222, 272 and 169 ppt wet weight after 14, 30 and 60 weeks of continuous TCDD promotion, respectively. These levels were approximately 10-fold higher than in serum; 21, 32 and 30 ppt wet weight after 14, 30 and 60 weeks of continuous TCDD promotion, respectively. The mean TCDD concentration in the lung, 30 weeks after cessation of TCDD promotion was 12 ppt wet weight, and was similar to that observed in animals receiving corn oil alone. TCDD concentrations in lung and serum where highly correlated (standard Pearson correlation statistic) and independent of age at onset of exposure, body and liver weight.

Summary

In summary this study shows that TCDD alone can promote the development of bronchiolar hyperplasia and AB metaplasia in the lung. In both cases the promotion of these lesions by TCDD was reversible. The main target region is the terminal bronchiole/alveolar duct junction where metaplasia is observed. Identification of the target cells in the lung is useful for further mechanistic studies.

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Table I. Time course and reversibility of lung lesions in female rats

	Alveolar Hyperplasia		Alveolar-Bronchiolar Metaplasia		Bronchiolar Hyperplasia
Group Name	Saline	DEN	Saline	DEN	DEN
I. Continuous					
C 14	0/6	0/6	0/6	0/6	0/6
C 30	0/6	2/6	0/6	0/6	0/6
C 60	0/6	8/11	0/6	1/11	0/11
T 14	0/6	1/6	0/6	0/6	0/6
T 30	0/5	2/6	0/5	0/6	0/6
T 60	0/8	8/14	6/8	6/14	6/14
II. Withdrawal					
C 30-16	0/6	1/6	0/6	0/6	0/6
T 30-16	0/6	1/6	2/6	1/6	0/6
T 30-30	1/9	8/11	0/9	0/11	1/11
III. Waiting					
T 0-16-14	---	2/6	---	0/6	0/6
T 0-16-30	---	2/15	---	1/15	0/15
IV. Waiting+withdrawal					
T 0-16-30-16	---	7/12	---	2/12	0/12

C, Corn oil; T, TCDD-treated; DEN, diethylnitrosamine.