STUDIES ON THE MECHANISM OF TCDD-INDUCED DEFORMITIES AND EMBRYO LETHALITY IN BIRDS

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Introduction

The toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds in birds has been well established in laboratory and field studies. Observed effects of TCDD and related chemicals in birds include developmental effects, reproductive failure, liver damage, wasting syndrome, and death. The mechanism of action of TCDD at the cellular level is mediated through the aryl hydrocarbon (Ah) receptor. However, the mechanism of toxic action at the organismal level is poorly understood. At present, there are several potential mechanisms to be considered (Figure 1).

For bird species, the most sensitive dose metric for dioxin-like chemicals are egg concentrations rather than adult tissue concentrations[1]. This is due in part to the stage-specific sensitivity of many species (birds, fish and mammals) during development and the relative tolerance of adults to the effects of dioxin-like chemicals. Thus, egg injection studies were conducted to determine if *in ovo* TCDD exposure could cause oxidative stress, using chicken eggs as a surrogate species.

In this study, we examined the role of radical oxygen species and mixed function oxidases (MFO) in the mechanism of TCDD-induced deformities and lethality by coinjecting radical scavengers and an MFO inhibitor (piperonyl butoxide). An alternative mechanism leading to terata (which was not examined in the current study) involves modulation of cell growth and differentiation, through interference with growth factor receptor signaling[2]. The main objectives of this work were to:

1. Investigate the probable mechanism(s) of deformities and embryo lethality in birds that have been exposed to Ah receptor agonists
2. Determine if *in ovo* TCDD exposure can cause oxidative stress in birds, using chicken eggs as a surrogate species
3. Determine if co-treatments with antioxidants, anti-inflammatory agents, or cytochrome P450 inhibitors can effectively antagonize any of the effects of TCDD on the developing chick

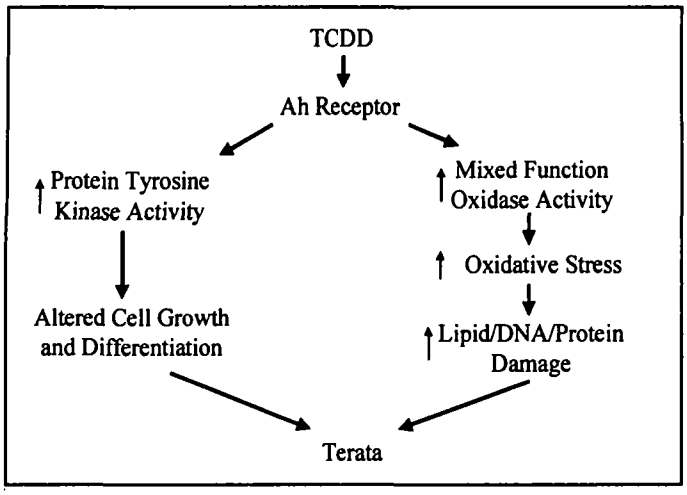

Figure 1. Possible mechanisms for TCDD-induced deformities

Materials and Methods

All biochemical reagents were purchased from Sigma. Procedures involving incubation and injection of whitehorn chicken (*Gallus domesticus*) eggs were adapted from Powell et.al. (1996)[3]. In brief, prior to injection, eggs were candled for the location of the air cell, weighed and allocated to treatment groups with equal weight distribution. Eggs were injected with TCDD (150 ng/kg which is approximately the LC50) or triolein (vehicle control), cotreatments and other treatments (see Table 1) prior to incubation (on day 0 of development) into the yolk. Eggs were then incubated in Petersime model #5 incubator (temperature 37.5 °C; humidity 65%). Eggs were candled on days 4, 11, and 17 and all nonviable eggs were opened and examined for the stage of development and any deformities. Hatchling chicks were weighed, euthanized, and then the liver, brain and heart were removed, weighed and frozen for biochemical analysis. The endpoints for which data were collected included

- survival,
- embryotoxicity,
- occurrence of abnormalities
- Physiological parameters:
  whole body, liver, brain and heart weight
- Biochemical parameters:
  Organs of interest:
  liver = major detoxification organ
  brain = lipid-rich tissue, highly susceptible to damage by reactive oxygen species
  EROD activity - fluorimetric measurement on 96 well microplates modified from Kennedy et.al. (1996)[4]
  oxidative DNA damage - measured as the ratio of 8OHdG/105dG: (modified from Shigenaga et.al. 1994)[5] – by HPLC/UV/Electrochemical detector

Table 1. Frequency of Abnormalities.

Treatment	# Abnormal/ # Fertile (% Abnormal)	Type of Effect
Sham	0/38 (0%)	
Control	0/37 (0%)	
TCDD	11/38 (28.9%)	neck edema, pipping muscle edema, Eye deformities, beak deformities, Liver necrosis, hemorrhage
Control + Piperonyl butoxide	1/15 (6.6%)	Yolk not completely absorbed Light feather color (all chicks)
TCDD + Piperonyl butoxide	1/15 (6.6%)	Subcutaneous (posterior) edema Light feather color (all chicks)
Control + Vitamin A	1/15 (6.6%)	Lower beak slightly deformed
TCDD + Vitamin A	2/15 (13.3%)	Neck edema, beak deformity, pipping muscle edema, hemorrhage
Control + Vitamin E	4/18 (22.2%)	Small underdeveloped or unopened eyes, pipping muscle edema, foot deformity
TCDD + Vitamin E	2/14 (14.2%)	skull deformity, small eyes
Control + SOD mimetic	0/10 (0%)	light color feathers
TCDD + SOD mimetic	1/6 (16.6%)	light color feathers, hemorrhage
Control + Piroxicam	0/14 (0%)	
TCDD + Piroxicam	2/11 (18.1%)	deformed legs, pipping muscle edema, hemorrhage
Epidermal Growth Factor	2/11 (18.1%)	deformed feet, head edema, edematous sac
Phenytoin	3/17 (17.6%)	deformed, crossed, fused beak, exencephaly, eye

Results and Discussion

All treatment groups with TCDD (except TCDD+Vitamin A), EGF, and control+PBO treatment groups caused significantly higher mortality than the control. None of the cotreatments significantly decreased mortality due to TCDD. On the contrary, TCDD+PBO significantly increased the mortality. Table 1 summarizes the abnormalities observed in different treatment groups. As "abnormal" counts any embryo with one or more types of developmental abnormalities – they are specified in the table. No abnormal embryos or hatchlings were observed in the sham or vehicle control groups. TCDD caused a significant increase in the incidence of developmental abnormalities. Non-normalized weights of whole body, liver, brain, heart and these organ weights normalized for body weights were compared between groups. There were no significant differences in the normalized data. Nonparametric ANOVA showed a significant decrease of non-normalized brain weight for TCDD, TCDD+vit.E and TCDD+piroxicam compared to control. No

differences between treatments were found for liver, heart or whole body weights. Significant induction was found in all treatments with TCDD regardless of the cotreatment compared to appropriate controls. Maximal induction reached about 50 fold over control. There was relatively high variation in EROD activity between individual chicks. None of the cotreatments showed effect on EROD activity compared to TCDD alone, only TCDD+vitamin E showed a slightly significant decrease.

None of the cotreatments provided significant protection against embryolethality caused by TCDD. PBO by itself or coinjected with TCDD increased the lethality compared to control or TCDD alone. EGF caused very high lethality, majority occurring in the early stage of development. TCDD cause an increase in the occurrence of abnormalities and decrease in brain weight compared to control. All TCDD treatments caused significant EROD induction in liver, slight decrease was caused by Vit.E cotreatment. There wasn't very high increase of oxidative damage in any treatments in liver and/or brain. None of the cotreatments could completely prevent the effects caused by TCDD

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