Heart morphology and liver EROD induction in Japanese quail embryos exposed to TCDD in ovo

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Introduction

Research in the past years has demonstrated that chlorinated dioxins and related halogenated hydrocarbons have significant impact on the developing cardiovascular system in birds. Exposure of avian embryos to dioxin is associated with subcutaneous, pericardial, and peritoneal edema[1,2], enlarged hearts[3,4], ventricular septal defects[4,5] and dilated ventricle cavities[6]. Walker and Catron[6] showed that PCB 126 induces a dose-related increase in heart weight in chicken embryos. Heid et al.[7] demonstrated the presence of the Ah receptor (AhR) and its co-receptor ARNT, in areas of the heart that are sensitive to TCDD-induced cardiotoxicity. In the same study a positive correlation between cardiotoxicity and the ability of halogenated aromatic hydrocarbons to activate the AhR pathway was shown. In the present study, Japanese quail (*Coturnix japonica*) embryos were used, and TCDD was injected into the yolk from where it is continuously absorbed via the yolk sac vessels thereby exposing the embryo throughout development. We studied the morphology and histology of embryonic hearts at day 8 of incubation and also investigated EROD activity in liver homogenates.

Material and Methods

Fertilized eggs from Japanese quail, weighing approximately 14 g, were purchased from Olstorps konservfabrik, Färgelanda, Sweden. TCDD was dissolved in a peanut oil/lecithin mixture (9:1, w:w) and then the oil/lecithin mixture was emulsified in water (1:1.5, v:v) by sonication (8,11). The emulsions were injected (20 µl) into the yolks of fertile eggs before incubation. Three groups of 30 eggs were injected with; vehicle only, low dose (1.5 ppb of TCDD) and high dose (15 ppb of TCDD). After injection, the eggs were incubated at 37.5°C and 60% relative humidity and turned every 6 h. The embryos were killed and the livers and hearts removed on day 8. Liver homogenates were prepared and EROD and protein assays were carried out as described in Brunström and Halldin[8]. Hearts were fixed in formalin and embedded in paraffin wax. Gross morphology and histology of the embryonic heart was studied by collecting transverse sections from the heart at levels representing specific morphological structures.

Results and discussion

Body weights, protein concentrations and EROD induction in liver are presented in Table 1. Comparing the three groups using ANOVA, significant difference was found for the EROD assay ($p<0.05$). As can be seen in Figure 1, embryos from the high dose showed the highest EROD induction. Both low and high dose exposed embryos exhibited EROD induction as compared to control embryos. No differences in protein concentration or embryo weights were found. No edema was found in any of the subjects.

The histopathological examination showed that the hearts from all groups were largely normal. Nuclei showed no alterations and the cells were normally differentiated. An overview of the hearts revealed a good differentiation between parts and connective tissues appeared normal. Heart morphology was quite variable regardless of treatment so it was not possible to detect minor differences between the groups. Heart size, dimensions of the heart sections, and ventricular or/and auricular measurements did not show any differences between the groups. The muscle tissue (myocardium) in the high dose group was, visually, less cohesive than in the control group. Also, these hearts exhibited an apparent cellular disorder. Despite these tendencies, the conclusion of the histopathological examination was that there were no obvious alterations in the hearts of the TCDD-treated embryos.

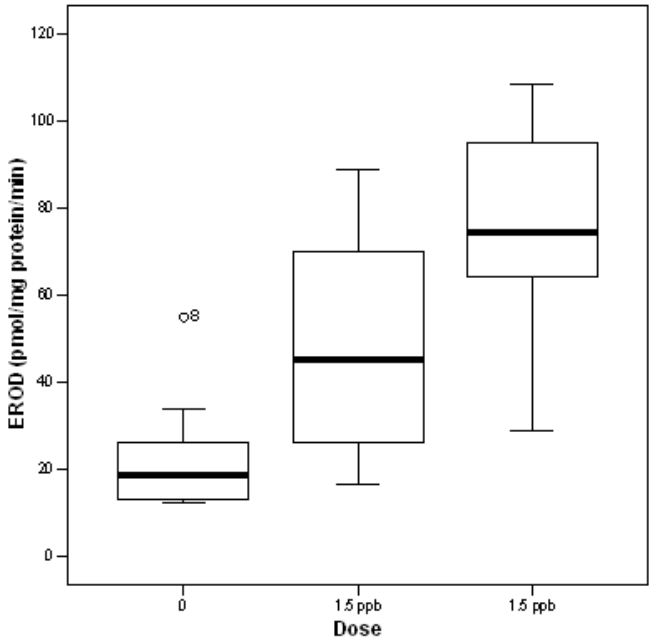
The results presented in this study are the first on Japanese quail. In birds, previous studies on *in ovo* exposure to TCDD have been performed with chicken. Walker and Catron[6] reported that TCDD injected on day 0 induced a dose-related increase in heart weight in White leghorn-babcock and Plymouth rock-barred embryos at day 10 of embryonic development. Plymouth rock-barred embryos were 4 to 5 times more sensitive to TCDD and exhibited a dose-related increase in left and right ventricle cavity area, consistent with dilated cardiomyopathy. Furthermore, TCDD induced a higher incidence of subcutaneous and peritoneal edema in a time-dependent way, indicative of heart failure. In our study, no symptoms of dilated cardiomyopathy were found at any of the doses used and no edema was evident. These results indicate that quail are less sensitive than chicken. However, TCDD cardiotoxicity in embryonic quails cannot be discarded since effects by TCDD have been shown to be time-dependent, and in this study only embryos in the middle of embryonic development were studied.

Interspecific differences in Ah receptor affinities for TCDD may partly account for species differences. It has previously been shown that Ah receptors prepared from turkey embryo livers were present in lower concentrations or had a lower affinity for TCDD than Ah receptors obtained from chicken embryo livers[9]. Sanderson and Bellward[10] found that the ED_{50} for EROD induction by TCDD was between one and two orders of magnitude lower in the chicken than in the domestic pigeon (*Columba livia*), great blue heron (*Ardea herodias*), and double-crested cormorant (*Phalacrocorax auritus*). Consistent with this, the apparent affinity of the hepatic cytosolic Ah receptor for TCDD was about an order of magnitude higher in the chicken than in the other species. In this study, induction of EROD activity in liver from TCDD exposed embryos was detected (Figure 1). However, quail embryos seem to be less sensitive than many other species to TCDD exposure. This is in agreement with Brunström and Halldin[8] who found that quails were 10 times less sensitive to TCDD, as compared to chickens, in terms of EROD induction *in vitro*.

Table 1. Embryo weight, EROD activity and protein content in liver of quail embryos at day 8 of incubation exposed to three TCDD concentrations (vehicle, 1.5 ppb, 15 ppb) injected before incubation.

Dose		Embryo Weight (g)	Protein (mg/liver)	EROD (pmol/mg protein/min)
0 ppb	**n**	12	12	12
	Average	0.79	0.73	22.26
	S.D.	0.09	0.21	12.25
1.5 ppb	**n**	12	12	12
	Average	0.77	0.71	48.64
	S.D.	0.06	0.18	24.07
15 ppb	**n**	12	12	12
	Average	0.76	0.68	74.43
	S.D.	0.09	0.19	25.90

Figure 1. EROD activity in liver of quail embryos at day 8 of incubation exposed to three TCDD concentrations (0, 1.5 ppb, 15 ppb) injected before incubation.



Acknowledgements

R. Merino is receipt from a Ph.D. fellowship from the Regional Government of Madrid (Spain). Authors would thank to B. Merino for her valuable help in histopathological interpretations of tissues.

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