ALTERATION OF CYP1A1, AHR AND ARNT mRNA EXPRESSION BY TCDD IN MOUSE PREIMPLANTATION EMBRYOS DEVELOPED FROM 8-CELL TO BLASTOCYST STAGE

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Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is one of the most potent environmental contaminants that lead to embryo loss, fetal development alteration and teratogenesis[1]. The effects of TCDD may occur at early embryogenesis[2, 3]. Most of the effects of TCDD are mediated by binding to aryl hydrocarbon receptor (AhR), subsequently heterodimerizing with aryl hydrocarbon receptor nuclear translocator (Arnt). The ligand-AhR-Arnt complex translocates to the nucleus where it regulates expression of genes including the cytochrome P450 1A1 gene (CYP1A1). However, the gene regulations by TCDD in preimplantation embryos remain unclear.

In preimplantation embryos, one of the key developmental transitions is embryonic compaction and differentiation, which occurs during development from 8-cell to blastocyst stage and is accompanied by changes in cell structure and developmental fate. Therefore, analysis of gene expression of AhR, Arnt and CYP1A1 in TCDD-exposed embryos at the 8-cell to blastocyst stage will be useful for identifying the stage-specific effect of TCDD on the preimplantation embryos.

In this study, we have used semiquantitative RT-PCR to examine gene expression of CYP1A1, AhR, and Arnt in the embryos after TCDD exposure during development from 8-cell to blastocyst stage.

Materials and Methods

Embryo Collection and TCDD Treatment

Animal experiments were performed according to the guideline on animal welfare at NIES. Female ICR mice (SLC, Japan), 9 to 10 weeks old, were superovulated with 5 IU pregnant mare serum gonadotropin (PMCG) followed by 5 IU human chorionic gonadotropin

(hCG) 48 h later. Superovulated females were mated with ICR males (SLC, Japan) for the collection of embryos. Mice were provided with food and water *ad libitum* and kept on a 12 light: 12 darkness cycle.

8-cell embryos were collected from oviduct at approximately 70 h after hCG treatment. TCDD (Combridge Isotope Laboratories, USA) was dissolved in DMSO (Wako, Japan) and added to M16 (Sigma, USA) medium. Embryos were cultured for 24 h in the M16 medium supplemented with various concentrations of TCDD (0, 0.01, 0.1, 1, 10, and 100 nM) under mineral oil in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C.

Semiquantitative RT-PCR

Total RNA was prepared from 40 embryos according to the acid guanidium phenol-chloroform method using an ISOGEN reagent (Nippon Gene, Japan)[4], and subjected to reverse transcription-PCR using 3'-Full RACE Core Set (TaKaRa, Japan) as previously described[5]. Yeast tRNA was used as a carrier. RQ1 DNase (Promega, USA) was added and the reaction mixture was allowed to incubate at 37 °C for 30 min to digest contaminating genomic DNA. For semiquantitative RT-PCR, rabbit globin mRNA was used as an internal standard to evaluate the efficiency of RNA extraction and reverse transcription.

PCR was performed using a set of primers summarized in Table.1. The reaction program in CYP1A1, AhR, Arnt, and globin consisted of 32 cycles (35 cycles in AhR and Arnt, and 28 cycles in globin,) of 94°C for 60s, 55 °C (60 °C in Arnt) for 60s, and 72 °C for 60s. Under the above conditions, the PCR reaction was conducted in the exponential range of amplification for each set of primers. The PCR products were separated by electrophoresis on 1.5% agarose gels, stained with ethidium bromide and photographed under a UV transilluminator (Image Saver AE-6905C, ATTO, Japan). The intensity of DNA bands was quantified by densitometry using Image Gauge V3.4 (Fujizi, Japan) software. The ratio of the value of density for the PCR products of CYP1A1, AhR, and ARNT to globin was calculated.

Statistical analysis

Experimental data were calculated using one-way analysis of variance (ANOVA) and Dunnett's test. A regression analysis was performed to measure the dose-response relationship. Statistical differences were considered significant at P value below 0.05.

Result and discussion

The induction of CYP1A1 is a major biochemical consequence of TCDD exposure through the AhR /Arnt complex, and is often used as a biomarker. In this study, six different dose groups of 8-cell embryos were cultured in medium containing 0, 0.01, 0.1, 1, 10, or 100 nM of TCDD for 24 h, and mRNA levels of CYP1A1, AhR and Arnt in embryos were measured at the end of incubation. Relative CYP1A1 mRNA levels were significantly higher in

TCDD-exposed (10 - 100 nM) groups than in control group (Fig.1A), and the highest level of CYP1A1 mRNA was observed in 100 nM TCDD exposed group, which was 7-fold higher than that in control group. In addition, there was a dose-dependent relationship between TCDD concentration and relative CYP1A1 mRNA level (r^2=0.95, $p < 0.01$), indicating that preimplantation embryos may respond to TCDD at gene regulation level.

The relative AhR and Arnt mRNA levels in all TCDD-exposed groups and control group were shown in Fig. 1B and 1C. No significant difference between exposure groups and control group was observed. These results demonstrate that TCDD does not greatly affect relative AhR and Arnt mRNA levels between 0 and 100 nM of TCDD.

In conclusion, the present study reveals that exposure of 8-cell embryos to TCDD for 24 h dramatically induces CYP1A1 mRNA expression in a TCDD dose-dependent manner. AhR and Arnt mRNA levels are not affected. To our knowledge, this is the first study on the alteration of gene expression in preimplantation embryos exposed to TCDD *in vitro*. Further study is needed to investigate the embryo and fetus development.

Reference

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Table 1 Primers Used for RT-PCR

Gene product	Upper (U) and lower (L) primer sequence	Amplification size (bp)	GenBank accession No.
CYP1A1	U 5'-CCTCTTTGGAGCTGGGTTTG-3'	230	NM009992
	L 5'-TGCTGTGGGGGATGGTGAAG-3'		
AhR	U 5'-CGCTGAAACATGAGCAAATTGG-3'	316	M94623
	L 5'-ACAGCTTAGGTGCTGAGTCACGG-3'		
ARNT	U 5'-GATGCGATGATGACCAGATGTG-3'	299	U10325
	L 5'-CAGTGAGGAAAGATGGCTTGTAGG-3'		
α-globin	U 5'-GCAGCCACGGTGGCGAGTAT-3'	256	V00875
	L 5'-GTGGGACAGGAGCTTGAAAT-3'		

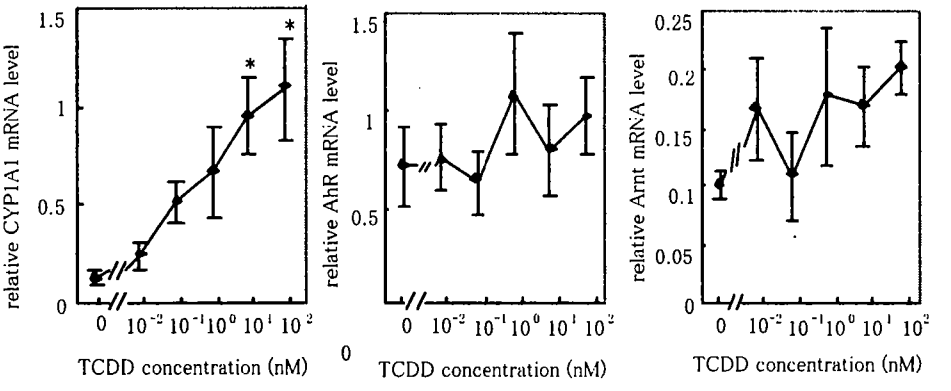

Fig.1 Alteration of gene expression in preimplantation embryos by TCDD. A: CYP1A1 mRNA. B: AhR mRNA. C: Arnt mRNA. Results are expressed as the mean±SE (n=3). * indicates $P < 0.05$, compared with DMSO-treated control