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Effect of 17β-estradiol on the expression of cytochrome P450 1A1 gene via an estrogen receptor dependent pathway in cellular and xenografted ovarian cancer models

Ryeo-Eun Go, Kyung-A Hwang and Kyung-Chul Choi*

Laboratory of Biochemistry and Immunology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk, Republic of Korea

Abstract

Introduction

Cytochrome P450 (CYP) 1A1 plays a major role in the metabolic activation of procarcinogens to carcinogens via aryl hydrocarbon receptor (AhR) pathway. In estrogen responsive cancers, 17β-estradiol (E2) may influence on AhR dependent expression of CYP1 family via the interaction between estrogen receptor (ER) and AhR. In the present study, the effect of E2/ER on the expression of AhR and CYP1A1 genes was investigated for BG-1 ovarian cancer expressing ER.

Materials and Methods

BG-1 cell was cultured with 0.1% DMSO (control), TCDD (10^{-8}M) and E2 (10^{-8}M). And to investigate the effects of TCDD and E2 on the RNA or protein expression of AhR or CYP1A1, RT-PCR or western blot were performed. Also, the xenografted mice transplanted with BG-1 ovarian cancer cells were ovariectomized for removal of endogenous estrogen and then were injected *i.p.* with TCDD, E2 or 0.1% DMSO in PBS during the experiment period (80 days). To evaluate the ability of TCDD or E2 to induce *in vivo* expression of AhR and CYP1A1, we performed an immunohistochemistry for AhR and CYP1A1 proteins on the tumor tissues obtained from the mice.

Results

In reverse transcription (RT)-PCR and western blot analysis, mRNA level of AhR was not altered, but its protein level was increased by TCDD or E2. The transcriptional and translational levels of CYP1A1 appeared to be increased by TCDD or E2. The increased expression of AhR and CYP1A1 induced by E2 was restored to the control level by the co-treatment of ICI 182,780, indicating that E2 induced the protein expression of AhR and CYP1A1 like TCDD via an ER dependent pathway. In an *in vivo* xenograft mouse model transplanted with BG-1 cells, the protein levels of AhR and CYP1A1 of tumor masses were also increased by E2 or TCDD.

Conclusion

These results indicate that E2 may promote AhR dependent expression of CYP1A1 via ER dependent pathway in BG-1 ovarian cancer expressing ER in the absence of TCDD, an agonist of AhR. The relevance of E2 and ER in CYP1A1 activation of estrogen responsive cancers may be targeted for developing more effective cancer treatments.

Result

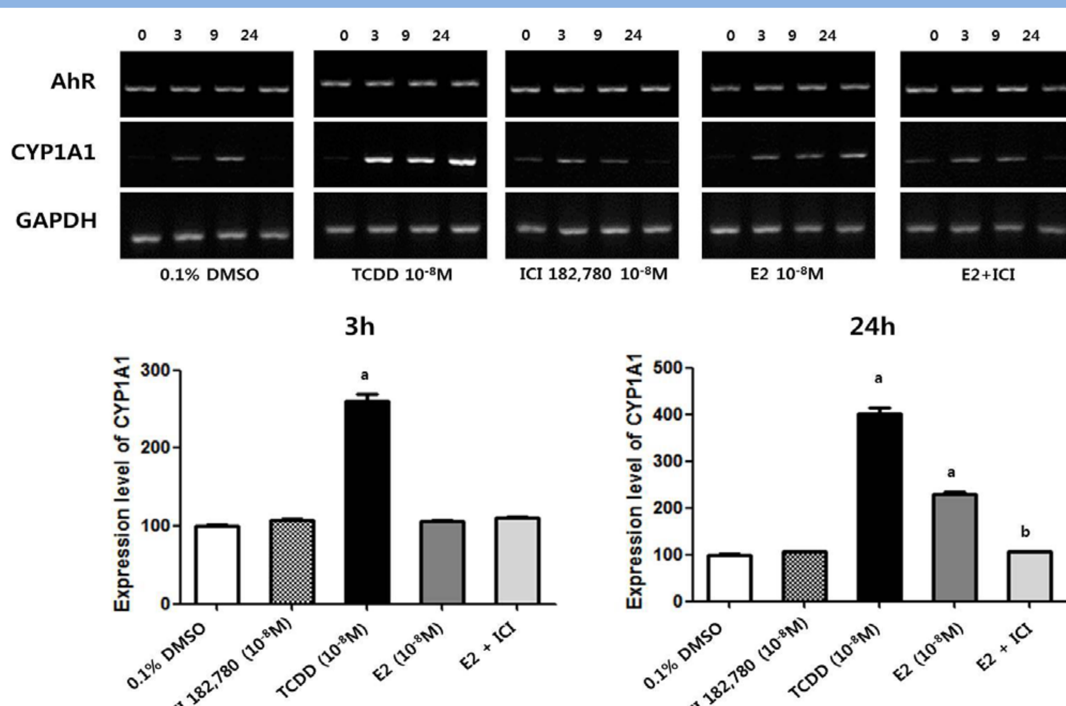

Figure 1. ***Altered mRNA expressions of AhR and CYP1A1 following the treatment with E2 or TCDD.*** BG-1 cells were seeded in 6-well plate and treated with 0.1% DMSO (a control), TCDD (a positive control, 10^{-8} M), ICI 182,780 (10^{-8} M), or E2 (10^{-8} M) in the presence or absence of ICI 182,780 (10^{-8} M) for 0, 3, 9, and 24 h. The band images of mRNA expression obtained from reverse transcription–RCR was quantified using Gel Doc 2000. Data represent the mean ± SD. a : Mean values were significantly different from the 0.1% DMSO (control), $P<0.05$. b : Mean values were significantly different from a single treatment of E2 (10^{-8} M), $P<0.05$ (Tukey's multiple comparison test).

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Result

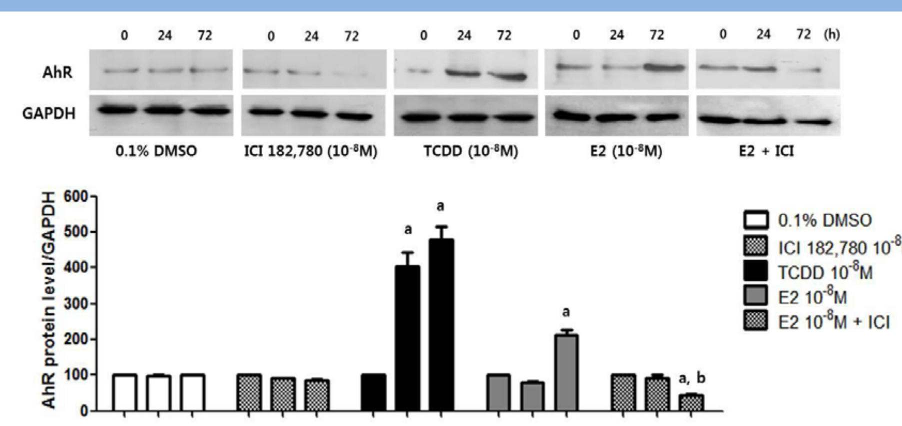

Figure 2. ***Altered protein expressions of AhR following the treatment with E2 or TCDD.*** BG-1 cells were seeded in 60-mm dish and treated with 0.1% DMSO (control), ICI 182,780 (10^{-8} M), TCDD (10^{-8} M), or E2 (10^{-8} M) in the presence or absence of ICI 182,780 (10^{-8} M) for 0, 24, and 72 h.

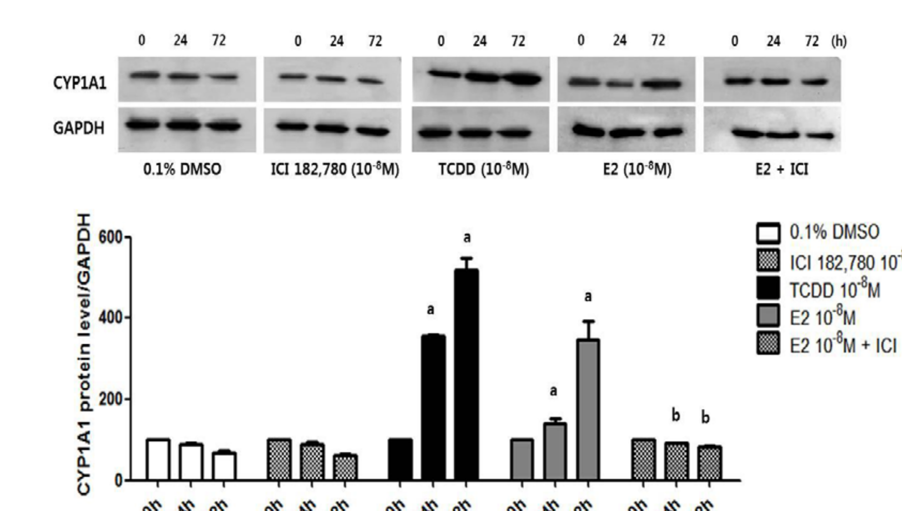

Figure 3. ***Altered protein expressions of CYP1A1 following the treatment with E2 or TCDD.*** BG-1 cells were seeded in 60-mm dish and treated with 0.1% DMSO (control), ICI 182,780 (10^{-8} M), TCDD (10^{-8} M), or E2 (10^{-8} M) in the presence or absence of ICI 182,780 (10^{-8} M) for 0, 24, and 72 h.

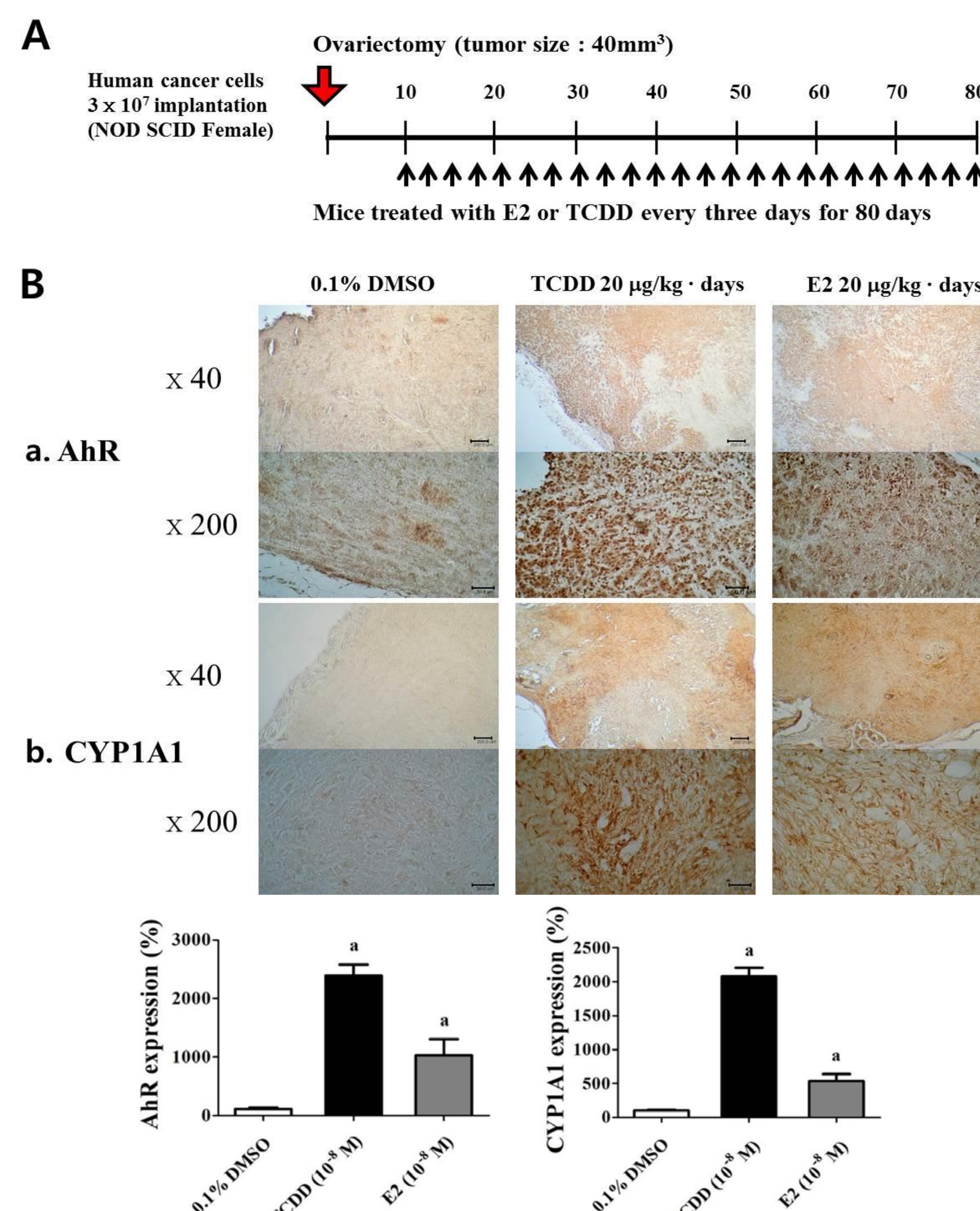

Figure 4. ***Representative immunohistopathological images of AhR, and CYP1A1 proteins in the isolated tumors.*** **(A)** The mice were injected *i.p.* with DMSO, E2, and TCDD every 3 days during experimental period. **(B)** At the end of the period, the tumor tissues were excised from each treatment group (0.1% DMSO, E2, and TCDD) of BG-1 ovarian cancer xenografted mice after sacrifice and then embedded in paraffin. Paraffin blocks were cut in into 5 μm thick sections and each section was treated with primary antibody by IHC staining protocol for measuring the immunohistological images of AhR **(a)** and CYP1A1 **(b)** (magnification, 40 x and 200 x). (**c**) The expression levels of AhR and CYP1A1 proteins were quantified by cell sens dimension software.

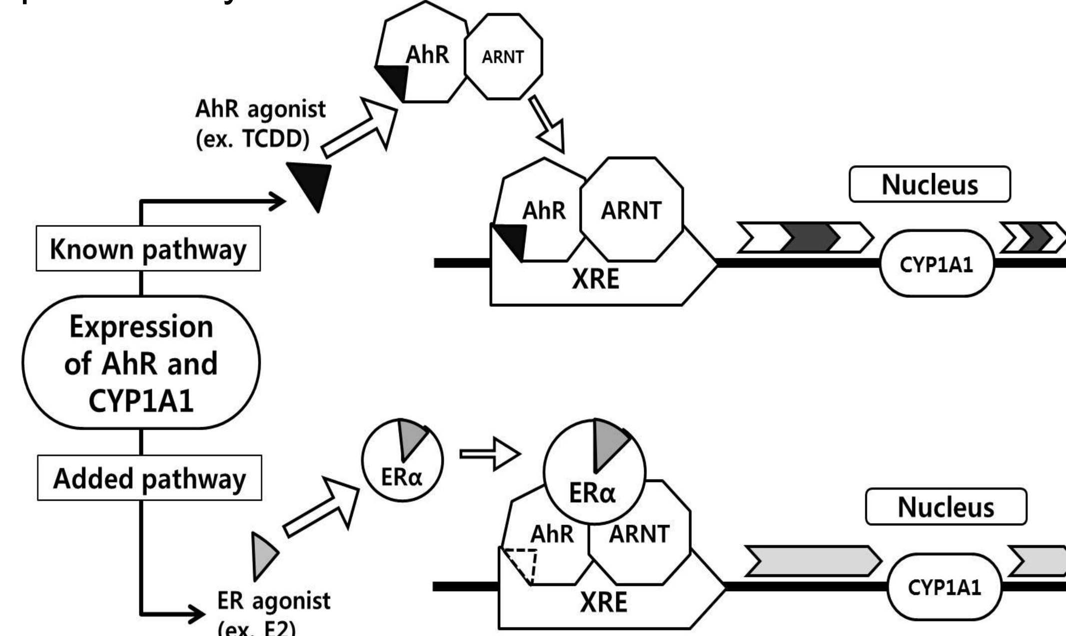

Figure 5. The cooperation of E2 and ER with AhR signaling in the induction of CYP1A1 expression in estrogen responsive BG-1 ovarian cancer cells. According to a well-known signaling pathway, ligand-bound AhR translocates from the cytoplasm into the nucleus and forms a complex by dimerizing with ARNT. This complex then binds to XREs and modulates the expression of CYP 1 family members including CYP1A1. In addition, the present study suggests that E2 may have the potential to induce the CYP1A1 expression via ER dependent-AhR signaling in estrogen responsive BG-1 ovarian cancer cells even though AhR is not charged with its agonist.

Conclusion

1. ***Effects of TCDD and E2 on the expression of AhR and CYP1A1 mRNAs***

To examine the effects of TCDD (a positive control) or E2 on the expression of AhR and CYP1A1 mRNAs, RT-PCR was performed for total RNAs extracted from TCDD or E2-treated BG-1 ovarian cancer cells. The mRNA expression of AhR was not changed by TCDD or E2, while the expression of CYP1A1 mRNA was significantly increased by E2 at 24 h and by TCDD at 3, 9, and 24 h compared to a control (0.1% DMSO) as shown in **Figure 1**. Because the expression of CYP1A1 was also induced by DMSO at 3 and 9 h, TCDD or E2-induced alteration in the mRNA expression of CYP1A1 was compared to DMSO treatment at each time point. In the co-treatment of ICI 182,780, E2-induced increase in the expression of CYP1A1 mRNA at 24 h was reversed to the control level (**Fig. 1**). These results indicate that E2 induced the increase in mRNA expression of CYP1A1 like TCDD via ER dependent pathway.

2. Effects of TCDD and E2 on the expression of AhR protein

To investigate the effects of TCDD and E2 on the protein expressions of AhR, western blot assay was performed for the proteins extracted from BG-1 ovarian cancer cells treated with TCDD or E2. The expression of AhR protein was significantly increased by the treatment of TCDD in a time-dependent manner compared to a control (0.1% DMSO) and by treatment of E2 for 72 h as shown in **Figure 2**. However, E2 induced-AhR protein expression was reversed to the control level by the co-treatment of ICI 182,780 at 72 h **(Fig. 2)**. These results indicate that E2 induced the expression of AhR protein like TCDD via an ER dependent pathway.

3. Effects of TCDD and E2 on the expression of CYP1A1 protein

To investigate the effects of TCDD and E2 on the protein expression of CYP1A1, western blot assays were performed for the proteins extracted from BG-1 ovarian cancer cells treated with TCDD or E2. The expression of CYP1A1 protein was significantly increased by treatment with TCDD for 24 and 72 h and by the treatment of E2 for 24 and 72 h compared to a control (0.1% DMSO) as demonstrated in **Figure 3**. However, the increased expression of E2 induced-CYP1A1 protein was significantly reduced by the co-treatment of ICI 182,780 for 48 or 72 h **(Fig. 3)**. These results indicate that the E2 induced the expression of CYP1A1 protein as did TCDD via an ER dependent pathway.

4. Immunohistochemistry for analysis of in vivo effects of TCDD and E2

The xenografted mice transplanted with BG-1 ovarian cancer cells were ovariectomized for removal of endogenous estrogen and then were injected *i.p.* with TCDD, E2 or 0.1% DMSO in PBS during the experiment period (80 days) according to a time schedule shown in **Figure 4A**. To evaluate the ability of TCDD or E2 to induce *in vivo* expression of AhR and CYP1A1, we performed an immunohistochemistry for AhR and CYP1A1 proteins on the tumor tissues obtained from the mice by using antibodies against these proteins. As a result, the protein expressions of AhR and CYP1A1 genes in the tissues of BG-1 ovarian cancer were considerably increased by the treatment of TCDD or E2 compared to the control, respectively **(Fig. 4B & C)**, which is similar to the *in vitro* results of western blot assay as shown in **Figure 2** and **3**.

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