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Ovarian cancer growth was induced by 2,3,7,8-tetrachlorodibenzo-ρ-dioxin through the regulation of CYP1A1 gene in an estrogen receptor-dependent pathway in BG-1 ovarian cancer cells

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Abstract

Environmental factors such as high meat consumption, caffeine, cigarette smoking, and endocrine disrupting chemical (EDCs) may enhance the risk of ovarian cancer. Cytochrome P450 (CYP) 1A1 may play a major role in metabolic activation of procarcinogens to carcinogens. 2,3,7,8-tetrachlorodibenzo-ρ-dioxin (TCDD) is a commonplace pollutant and a promoter of carcinogenesis as the most potent substance. In this study, we examined the effects of TCDD in the presence of 17beta-estradiol (E2) on the expressions of CYP1A1, CYP1B1, and aryl hydrocarbon receptor (AhR) by RT-PCR and western blot analysis. In addition, the cell viability by TCDD and E2 was examined in BG-1 human ovarian cancer cells by MTT assay. To evaluate the cell viability, BG-1 cells were cultured with control (0.1% DMSO), E2 (1×10^{-9} M) or TCDD (10^{-6}-10^{-8} M). E2 markedly increased BG-1 cell viability about 5 times and TCDD also induced BG-1 cell viability the most at 1×10^{-8} M compared to control. When co-treated with ICI 182,780, an ER antagonist, BG-1 cell viability was reversed to the level of control. Although mRNA expression of CYP1B1 or AhR was not altered by E2 or TCDD, the transcriptional level of CYP1A1 appeared to be increased by E2 or TCDD in a time-dependent manner. Furthermore the translational level of AhR and CYP1A1 appeared to be increased by E2 or TCDD in a time-dependent manner. In xenografted mouse models transplanted with BG-1 cells, E2 treatment significantly increased the tumor mass formation about 6 times and TCDD induced tumor formation about 4 times compared to vehicle (0.1% DMSO) during 80 days. In addition, expression levels of proliferation cell nuclear antigen, AhR and CYP1A1 are increased in E2 or TCDD-treated tumor section compared to the control. Taken together, TCDD may induce ovarian cancer cell growth via CYP1A1 gene expression and have a disruptive effect in ER expressing cells or tissues.

Key words: **2,3,7,8-tetrachlorodibenzo-ρ-dioxin; endocrine disruption; estrogen receptor; cytochrome P450; ovarian cancer cells**

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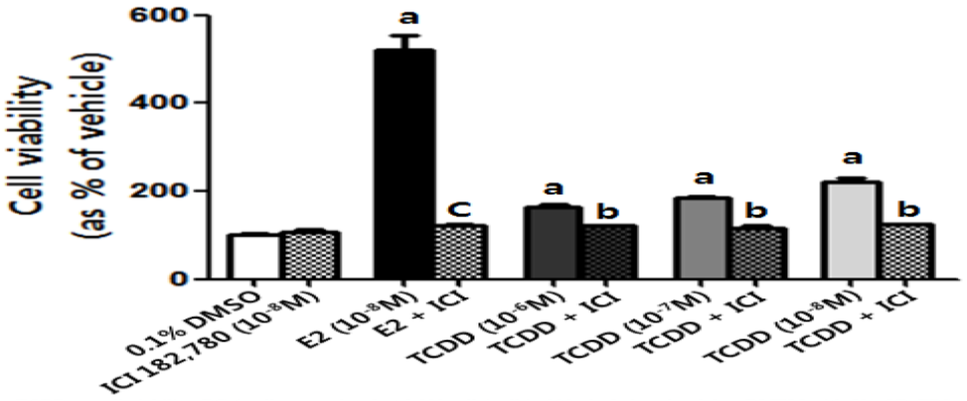
Result



TCDD on cell viability, BG-1 cells were plated at 3,000 cells/well in 200 μl of phenol red-free DMEM with 5% CD–FBS medium. Cells were cultured in phenol red-free DMEM with vehicle (0.1% DMSO, control), E2 (10^{-8} M), TCDD (10^{-6}-10^{-8} M) in the present or absence ICI 182,780 (10^{-8} M) for 9 days. The cell viability was measured using an MTT assay. Values are the means ± SD. a : Mean values were significantly different from 0.1% DMSO (vehicle), *P*<0.05. b : Mean values were significantly different from TCDD 10^{-8} M, *P*<0.05. c: Mean values were significantly different from E2 10^{-8} M, *P*<0.05. (Dunnett's multiple comparison test).

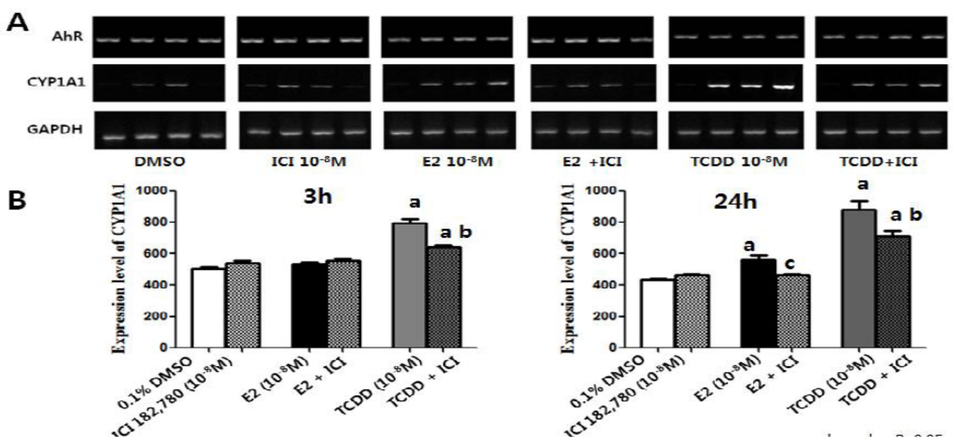


Figure 2. *Altered mRNA expressions of AhR and CYP1A1 following the treatment with E2 or TCDD.* BG-1 cell were seeded in 6-well plate and treated with 0.1% DMSO (negative control), E2 (10^{-8} M), TCDD (10^{-8} M) i the present or absence ICI 182,780 (10^{-8} M) at 0, 3, 9, and 24 h. The level of mRNA expression obtained fro reverse transcription–RCR (A) was quantified using Gel Doc 2000 (B). Data represent the mean ± SD. a Mean values were significantly different from 0.1% DMSO (vehicle). b : Mean values were significantl different from TCDD 10^{-8} M. c : Mean values were significantly different from E2 10^{-8} M, *P*<0.05. (Turkey multiple comparison test).

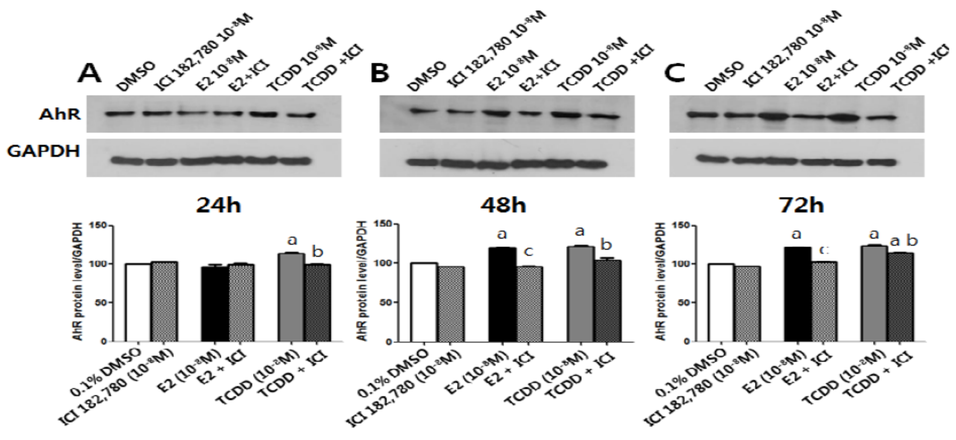


Figure 3. Altered protein expressions of AhR following the treatment with E2 or TCDD. BG-1 cells were seeded in 60-mm dish and treated with 0.1% DMSO (control), E2 (10-8 M), and TCDD (10-8 M) in the present or absence ICI 182,780 (10-8 M) at 24 (A), 48 (B), and 72 h (C). Quantification of proteins of AhR and GAPDH were conducted by scanning the densities of bands on a transfer membrane using Gel Doc 2000. a : Mean values were significantly different from the 0.1% DMSO (vehicle), P<0.05. b : Mean values were significantly different from TCDD 10-8 M, P<0.05. c : Mean values were significantly different from E2 10-8 M, P<0.05. (Turkey's multiple comparison test).

Result

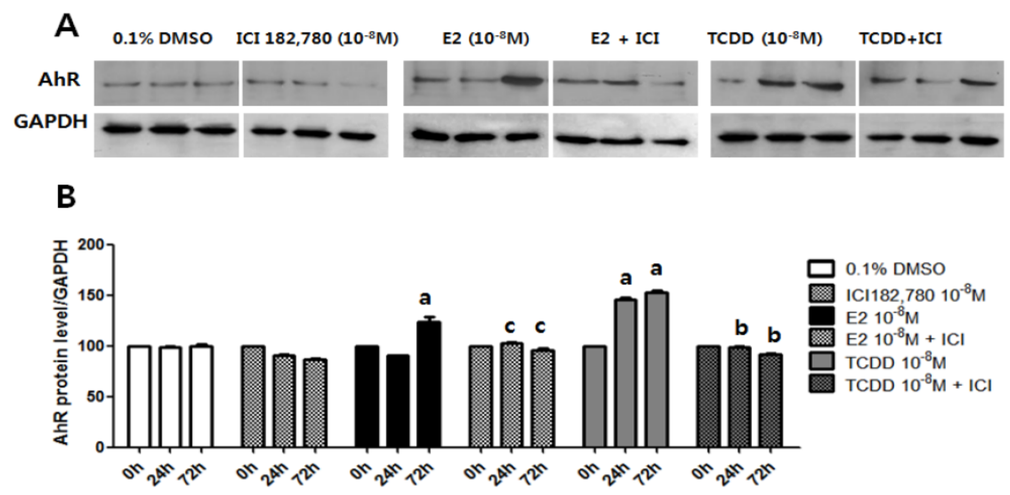


Figure 4. Altered protein expressions of AhR following the treatment with E2 or TCDD in a time-dependent manner. BG-1 cells were seeded in 60-mm dish and treated with 0.1% DMSO (control), E2 (10-8 M), TCDD (10-8 M) in the present or absence ICI 182,780 (10-8 M) in a time-dependent manner (A). Quantification of proteins of AhR and GAPDH were conducted by scanning the densities of bands on a transfer membrane using Gel Doc 2000 (B). a : Mean values were significantly different from the 0.1% DMSO (vehicle), P<0.05. b : Mean values were significantly different from the TCDD 10-8M, P<0.05. c : Mean values were significantly different from the E2 10-8 M, P<0.05. (Turkey's multiple comparison test).

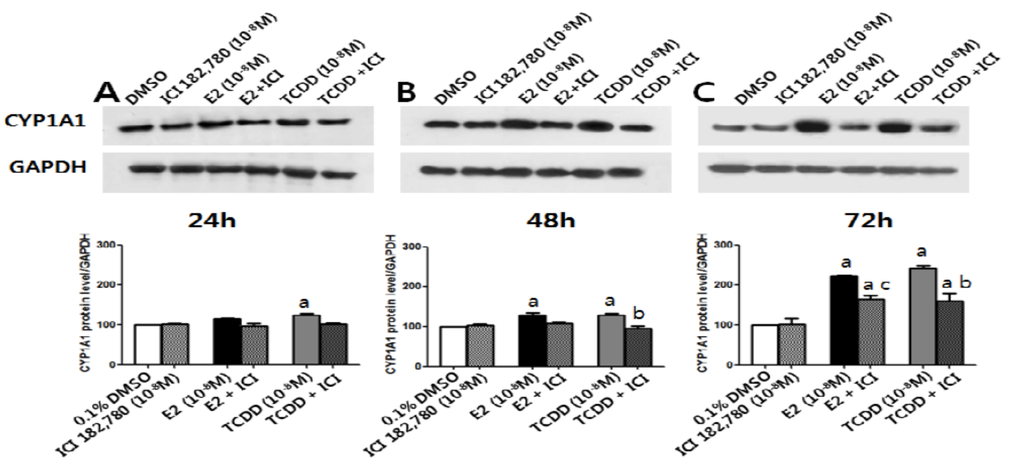


Figure 5. Altered protein expressions of CYP1A1 following the treatment with E2 or TCDD. BG-1 cells were seeded in 60-mm dish and treated with 0.1% DMSO (control), E2 (10-8 M), and TCDD (10-8 M) in the present or absence ICI 182,780 (10-8 M) at 24 (A), 48 (B), and 72 h (C). Quantification of proteins of CYP1A1 and GAPDH were conducted by scanning the densities of bands on a transfer membrane using Gel Doc 2000. a : Mean values were significantly different from 0.1% DMSO (vehicle). b : Mean values were significantly different from TCDD 10-8 M. c : Mean values were significantly different from E2 10-8 M, P<0.05 (Turkey's multiple comparison test).

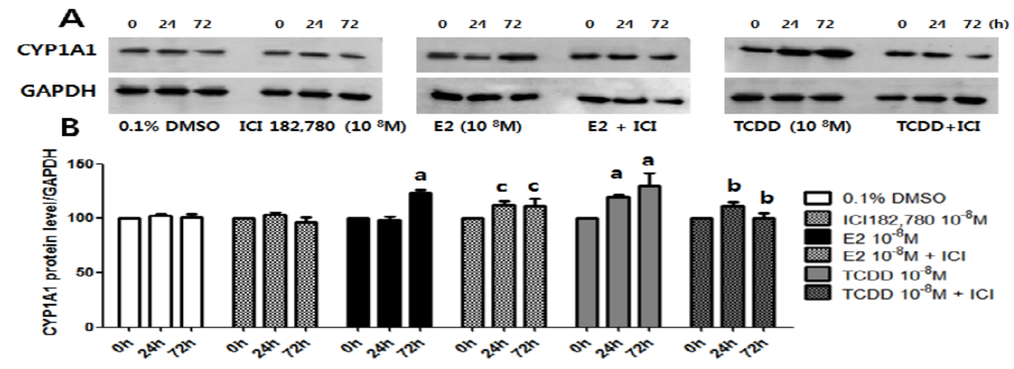


Figure 6. Altered protein expressions of CYP1A1 following the treatment with E2 or TCDD in a time-dependent manner. BG-1 cells were seeded in 60-mm dish and treated with 0.1% DMSO (control), E2 (10-8 M), TCDD (10-8 M) in the present or absence ICI 182,780 (10-8 M) in a time-dependent manner (A). Quantification of proteins of CYP1A1 and GAPDH were conducted by scanning the densities of bands on a transfer membrane using Gel Doc 2000 (B). a : Mean values were significantly different from 0.1% DMSO (vehicle), P<0.05. b : Mean values were significantly different from TCDD 10-8 M, P<0.05. c: Mean values were significantly different from E2 10-8 M, P<0.05. (Turkey's multiple comparison test).

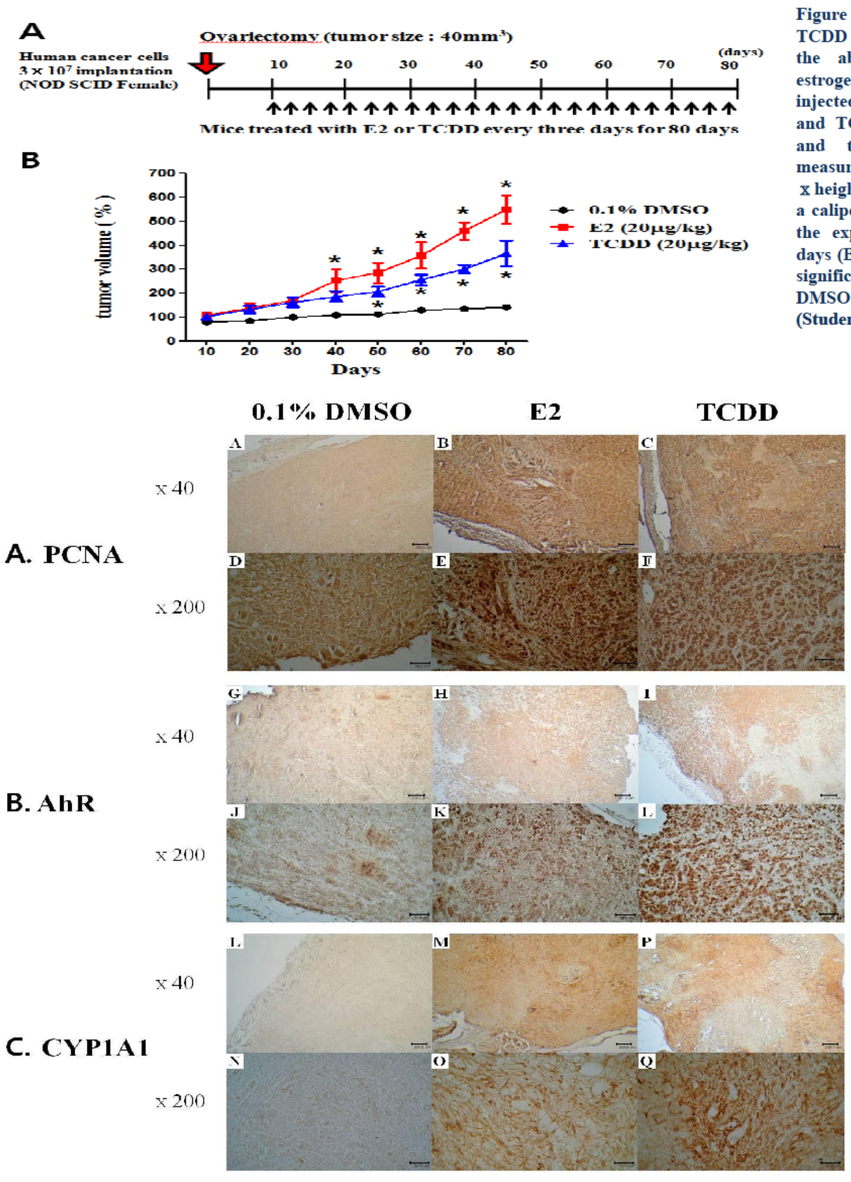


Figure 7. Effect of E2 and TCDD on the tumor growth in the absence of endogenous estrogen. The mice were injected i.p. with DMSO, E2, and TCDD every 3 days (A) and tumor volumes were measured by length x width x height x 0.5236 (mm3) using a caliper every 10 days during the experiment period of 80 days (B). * : Mean values were significantly different from DMSO (vehicle), P<0.05. (Student's t-test).

Figure 8. Representative immunohistopathological images of PCNA, AhR, and CYP1A1 proteins in the isolated tumors. 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 PCNA (A), AhR (B), and CYP1A1 (C). (magnification, 40 x and 200 x).

Conclusion

1. In a cell viability assay, the treatment of E2 or TCDD induced a significant proliferation in BG-1 ovarian cancer cells. When ICI 182,780 was treated with E2 or TCDD, E2 or TCDD-induced cell proliferation was reversed as much as the control (0.1% DMSO). These results provide that TCDD induced BG-1 ovarian cancer cell proliferation via ER dependent pathway, as did E2.

2. In a RT-PCR analysis, the treatment of E2 or TCDD induced the increased mRNA expression of CYP1A1, which was also reduced completely or partially by the co-treatment of ICI 182,780. By the way, there were no alterations in the expression level of AhR mRNA by the treatment of E2 or TCDD.

3. The exposure of E2 or TCDD in BG-1 cancer cells induced the increased expression of both AhR and CYP1A1 proteins in a time-dependent manner, which was also reduced completely or partially by the co-treatment of ICI 182,780. These results suggest that TCDD induced the increased gene expression of AhR and CYP1A1 in mRNA or protein level via ER dependent pathway in BG-1 ovarian cancer cells.

4. These in vitro results were confirmed by in vivo animal models using the ovariectomized mice exposed to E2 or TCDD. E2 and TCDD stimulated the BG-1 ovarain tumor growth and increased the protein expressions of PCNA, AhR, and CYP1A1 compared with the control (0.1% DMSO) in vivo. As a processivity factor for DNA polymerase δ and an essential factor of DNA replication, PCNA has been used as a cell proliferation marker and its expression is known to be mediated via ER pathway in estrogen responsive cancers.

5. These results also demonstrate that E2 and TCDD may promote the growth of BG-1 ovarian cancer and the protein expressions of AhR and TCDD in vivo in the absence of endogeneous estrogen, which are the similar results with the ones of in vitro assays.

6. In conclusion, the present study shows that both E2 and TCDD may have the capability of proliferating BG-1 ovarian cancer and stimulating the protein expression of AhR and CYP1A1 via ER dependent pathway.

7. In addition, this study also provides that ER signaling pathway is related with AhR signaling pathway and TCDD may have a potential estrogenic activity to promote the proliferation of ER expressing cancer cells by affecting this connection.

8. Based on these results, further studies are needed to elucidate the close relationship between ER and AhR pathways and its importance in estrogen responsive cancers for developing more effective cancer treatments targeting this process.

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