Dioxin Alters Expressions of Growth Regulatory Factors in Human Prostate Cells in Culture

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Introduction

Carcinoma of the prostate is the most commonly diagnosed cancer in American men and is second only to skin cancer in cancer death in males (1). Although the prostate cancer is such an urgent public health problem, mechanism and etiology of the carcinoma still remains unclear. Since growth factors are potent modulators of cellular proliferation, differentiation and cell death in the prostate, they are suggested to be associated with the carcinogenesis process of the prostate (2). Altered regulation of growth regulatory factors such as TGF-α, TGF-β_1, bFGF, EGF, etc. was observed in the tissues of prostate cancer and benign prostatic hyperplasia (BPH) (3). Thus, it has been suggested that alteration of growth regulatory factors may be one of plausible mechanisms of the hyperplasia or carcinoma of the prostate. Because TCDD is a potent dysregulator of growth regulatory factors (4), it would be interesting to see if TCDD plays any role in altering growth regulatory factors in human prostate cells, thereby leading to the prostate diseases such as carcinoma and hyperplasia. However, due to the limited prostate *in vitro* system, there has been lack of data on the effects of TCDD in human prostate cells. Therefore, the present study has attempted to examine possible effects of TCDD on alteration of growth regulatory factors in human prostatic epithelial cells.

Material and Methods

Cell cultures and chemical treatment: Human primary epithelial cells immortalized with transfection of SV40 negative origin plasmid were used for the experiments (5). Cells were maintained at 37 ^{0}C, 5% CO_2 incubator. The culture media was the complete K-SFM medium (Gibco-BRL, Gaithersburg, MD) which contains 50 μg/ml of bovine pituitary extract and 5 ng/ml EGF, plus antibiotic/antimyotic mixture (penicillin 100 U/ml, streptomycin 100 μg/ml and fungizone, 25 μg/ml). Cells were passaged upon confluence at 1 to 3 ratio. All

experiments were conducted on the cells at passage 30. Cells at 70% confluency were treated with 0.1, 1, 10, 100 nM TCDD for 24 hr. Control cells were treated with 0.1% DMSO only.
mRNA stability assay: Cells were culture as described above and treated with 10 nM TCDD. After incubation for 12 hr, medium was removed from control or TCDD-treated cultures and pooled. Actiniomycin D (5 μg/ml) was added to the pooled medium to block new transcription initiation and the medium was then added back to the culture. At the indicated time after addition of actinomycin D, total RNAs were prepared for RT-PCR analysis.
RT-PCR analysis: Total RNAs were prepared from RNA isolation kit, according to the manufacturer's instruction followed by RNase-free DNase I digestion (6). PCR primers were synthesized with Applied Biosystem DNA synthesizer and purified with NP-5 column. cDNA was synthesized with reverse transcriptase and an aliquot of the radiolabelled PCR products were loaded on the 10% PAGE and the respective bands were analyzed by the image analyzer (Bio Rad, USA). Densitometric values of mRNA were normalized to GAPDH.

Results and Discussion

A marked elevation of TGF-β_1 is frequently observed in both BPH and prostate cancer. Its elevation is associated with tumor cell growth, extracellular matrix turnover and suppression of immune system (7). The present study revealed a dose-dependent increase of TGF-β_1 mRNA with exposure to TCDD (Table 1.). Since increase of TGF-β_1 is closely associated with the prostate cancer, altered expression of the growth factor by TCDD exposure may be implicated with the carcinogenic process of the prostate cells. TCDD has been shown to downregulate hepatic and/or uterine estrogen receptor in rodent species (8). However, there is no data available on antiestrogenic effects of TCDD in human prostate cells. The results showed a dose-dependent decrease of estrogen receptor (ER-1) mRNA following TCDD exposure to human prostate cells *in vitro*, indicating a potent antiestrogenic effects of TCDD in human prostate cells. Thus, it may be postulated that imbalance between androgenic and estrogenic effects by TCDD may lead to the uncontrolled growth of prostate cells, which ultimately may contribute to the development of the hyperplasia or carcinoma. In the current study, mRNA levels of *Ah* receptor were decreased with an increase of TCDD concentrations. The results indicate that *Ah* receptors are present in the human prostaic epithelial cells and the receptors may be involved in the mediation of gene expressions observed in the study. Both PAI-2 and IL-1β are known dioxin-responsive genes. Regulation of PAI-2 is closely associated with cell migration, proliferation and differentiation (9). IL-1β is a potent regulator of T lymphocyte functions and TCDD acts on immunocompetent cells as a suppressant (10). Thus, modulation of PAI-2 or IL-1β expression by TCDD may affect the normal function of immune system and growth regulations. The result showed increases of PAI-2 and IL-1β mRNA levels up to 1.6 and 1.5 fold, respectively. While regulations of PAI-2 and IL-1β are well established in a human squamous carcinoma cell line (11), responses of these genes with exposure to TCDD are not known in human prostate cells. This study demonstrated a first evidence that these genes are expressed dose-dependently by TCDD. When cells were treated with actinomycin D to examine mRNA stability, mRNA levels of PAI-2 from cells treated with TCDD and actinomycin D showed a slower time-dependent decay, as compared to the cells treated with actinomycin D only (data not shown). The results suggest that regulation of PAI-2 in prostate cells in response to TCDD may be under post-transcriptional

control. mRNA expression of ER-1 and TGF-β_1 in the cells treated with 10 nM TCDD for 24 hr was increased in a time-dependent fashion (Table 2.). Maximum responses of ER-1 and TGF-β_1 were observed 24 hr and 12 hr after the exposure, respectively. This study is the first attempt ever reported to analyze effects of growth regulatory factors in the human prostatic epithelial cells in response to TCDD exposure. Given that dioxin responses are tissue- and species- specific (12), the results obtained from the human prostate cells are believed to be a valuable information for understanding effects of TCDD in human prostate and improving risk assessment of TCDD toxicity in humans. In addition, since the human *in vitro* system used in the present study is prostatic epithelial cells, from which the cell type BPH and prostate cancer are mostly originated, the present findings may provide additional advantage in understanding carcinogenesis process of the prostate. While link between TCDD exposure and diseases of prostatic cells is not well established, the present results from human prostate cells may help understand biochemical mechanism of a possible TCDD-induced prostatic effects in humans and provide scientific basis for further studies (including epidemiological studies) to elucidate association between TCDD exposure and human prostate diseases such as prostate cancer and BPH.

Table 1. Dose -dependent alterations of mRNA levels in human prostate cells treated with TCDD for 24 hr. Densitometric traces were normalized to GAPDH. Values are ratio to the control cells treated with DMSO only. Means and S.D. from three separate experiments were presented.

Dose(nM)	0.1	1	10	100
TGF-β_1	1.31±0.34	1.74±0.25	2.50±0.72	3.64±1.24
ER-1	0.95±0.08	0.71±0.12	0.61±0.13	0.19±0.14
AhR	0.94±0.12	0.91±0.15	0.81±0.12	0.75±0.14
PAI-2	0.95±0.14	1.35±0.15	1.58±0.18	1.63±0.08
IL-1β	1.24±0.08	1.15±0.05	1.35±0.07	1.47±0.05

Table 2. Time-dependent alteration of ER-1 and PAI-2 mRNA levels following 10 nM TCDD exposure. Values were calculated as described in the legend of table 1.

Time(hr)	3	6	12	24
ER-1	0.72±0.15	0.64±0.18	0.58±0.14	0.52±0.21
TGF-β_1	1.35±0.35	1.89±0.54	2.84±0.52	2.68±0.85

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