Regulation of Dioxin-responsive Genes in Human Endometrial Cells

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Abstract

Human endometral cells were treated with various concentrations of TCDD. RT-PCR analysis showed dose-dependent increases of PAI-2 and IL-1β in human endometrial cells. Treatment of cells with CHX showed substantial increases of PAI-2 and IL-1β. mRNA stability assay showed a slower, but statiscally insignificant, decay of PAI-2 mRNA in TCDD-treated cells. The present study revealed that PAI-2 and IL-1β are responding to TCDD in a dose-dependent manner and these genes are under positive transcriptional control.

Introduction

Concerns over the endometrial effects by dioxin exposure has ever increased since a recent report on a link between TCDD exposure and severe endometriosis in rhesus [1] monkey. Endonetriosis is the disease associated with chronic pain and infertility, affecting about 10 % of reproductive age women[2]. The recent increases of the disease is associated with urban life in the industrialized area and the trend of increase coincides with the increased production of PCBs and the related dioxin pollution since World War II[3]. It is suggested that possible mechanisms of the disease may be related with immune dyfunction such as altered cytokine production, increase of TNF, and decrease of IL-6[4-6]. However, molecular mechanisms of endometrial effects with exposure to TCDD is still unclear. Thus, the present study has attempted to use human endometrial cells to study responses and regulation of dioxin-responsive genes such as PAI-2 and IL-1β.

Experimental Methods

Cell culture: Human endometrial stromal cells immortalized by temperature-sensitive SV40 T-antigen (a genrous gift from Dr. Kaufman, U.S.A.) were used for the study[7]. Cells were maintained in 5 % CO_2 incubator at 33 °C. Culture media consist of the 1:1 mixture of F-12 and I-199 with 1 % FBS, 3 % BCS, glutamine, insulin and antibiotics.

Chemical treatment: Cells were treated with 0.1 % DMSO, 0.1, 1, 10, 100 nM TCDD for 24 hrs. For mRNA stability assay, actinomycin D (5 μg/ml) was used to block new transcription initiation. mRNA was isolated from the cells, 1, 3, 6, 12 hrs after actinomycin treatment. To inhibit protein synthesis, Cycloheximide (CHX) (10μg/ml) was added to the media in absence or presence of TCDD (10nM).

RT-PCR analysis: Total RNAs were prepared froma RNA isolation kit, according to the manufacturer,s instruction followed by Rnase-free Dnase I. PCR primers were synthesized with Applied Biosystem DNA synthesizer and purified with NAP-5 column. cDNA was synthesized with reverse transcriptase and an aliquot of the synthesized cDNA was used as a template for PCR as described previously[8]. Radiolabeled PCR products were loaded on the 10 % PAGE and the bands were analyzed by the image analyzer (Bio Rad, U.S.A.). The densitometric values of mRNA were normalized to GAPDH.

Results and Discussion

PAI-2 is a dioxin-responsive gene and its regulation is closely associated with cell migration, proliferation and differentiation[9]. A recent report indicates a close link between PAI-2 mRNA increases and endometrial cancer progression[10]. RT-PCR analysis revealed upto 2 fold increase of PAI-2 mRNA levels (Table 1). This is an evidence that human endometrial cells respond with TCDD exposure in a dose-dependent manner. Cells treated with CHX in presence or absence of TCDD showed 2 fold increase of PAI-2 mRNA levels. PAI-2 mRNA levels from the cells treated with CHX alone were similar to the cells treated with CHX plus TCDD. mRNA levels from the cells treated with TCDD for 24 hrs and followed by actinomycin D treatment showed a slower, but statistically insignificant, time-dependent decay, as compared to the cells treated with actinomycin D alone. IL-1β is a dioxin-responsive gene and produced by mononuclear phagocytes[11]. RT-PCR analysis showed a dose-dependent increase of IL-1β mRNA over the doses used (Table 1). mRNA level of IL-1β in the cells treated with CHX showed 1.8 forld increase, relative to the control cells. Cells treated with CHX plus TCDD showed a similar level of IL-1β mRNA, as compared to the cells treated with CHX only. mRNA stability assay showed a similar rate of RNA decay between TCDD-treated and control cells (data not shown). It is interesting to note that CYP1A1 mRNA levels was not significantly induced in human endometrial cells treated with TCDD for 24 hrs. The present study revealed that the transcriptional levels of PAI-2 and IL-1βrespond withexposure to TCDD in dose-dependent manner and are under positive transcriptional control. Considering a great deal of interspecies difference in dioxin responses, the results obtained from human endometrial cells can

be a valuable information for understanding mechanism of dioxin toxicity in humans which will lead to a better assessment of human risks from the dioxin exposure..

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Table 1. Dose-dependent increases of PAI-2 and IL-1β mRNA levels in human endometrial cells treated with TCDD for 24hrs. Denstrometric traces were normalized to GAPDH. Values are percent increase as compared to the control cells treated with 0.1% DMSO only.

Dose (nM)	0.1	1	10	100
PAI-2	160± 8	190±15	220±22	180±48
IL-1β	120±33	120±42	140±25	180±36

References

(1) Rier, S. E.; Martin, D.C.; Bowman, R.E.; Dmowski, W.P.;Becker, J.L. *Fund. Appl. Toxicol.*, 1993, **21**, 433-441.
(2) Vessey, M. P,; Villard-Mackintosh, L.; Painter, R. *BMJ*, 1993, **306**, 182-184.
(3) Koninckx, P. R.; Braet, P.; Kennedy, S. H.; Bavlow, D. H. *Human Rep.*, 1994, **9**, 1001-1002.
(4) Berchuck, A.; Boyd, J. *Cancer*, 1995, **76**, 2034-2040.
(5) Whitworth, C. M.; Mulholland, J.; Dunn, R. C.; Glasser, S. R. *Fertil. Steril.*, 1994, **61**, 91-96.
(6) Guidice, L. C.; Dsupin, B.A.; Irwin, J.C. *J. Clin. Endocronal. Metab.*, 1994, **79**, 1284-1293.
(7) Rinehart, C. A.; Haskill, J. S.; Morris, J.S.; Butler, T. D.; Kaufman, D. G. *J. Virol.*, 1991, **65**, 1458-1462.
(8) Doehr, O.; Vogel, C.; Abel, J. *Arch. Biochem. Biophys.*, 1995, **321**, 405-412.
(9) Vassalli, J. D.; Sappino, A. P.; Belin, D. *J. Clin. Invest.*, 1991, **88**, 1067-1072.
(10) Gleeson, N. C.; Gonsalves, R.; Bonnar, J., *Cancer*, 1993, **72**, 1670-1672.
(11) Dinarello, C. A., *Blood*, 1991, 1627-1652.