EFFECT OF ANDROGEN ON EXPRESSION OF ARYLHYDROCARBON RECEPTOR-MEDIATED GENES

Sanada N[1,2], Okamura K[2], Kizu R[2]

[1]Division of Pharmaceutical Sciences, Kanazawa University Graduate School of Natural Science and Technology, Kanazawa 920-1192, Japan; [2]Faculty of Pharmaceutical Sciences, Doshisha Women's College of Liberal Arts, Kyotanabe 610-0395, Japan

Introduction

A number of aryl hydrocarbons (Ahs) including dioxins and polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants and potent Ah receptor (AhR) ligands. They elicit a diverse spectrum of biological and toxic responses, which include tumor promotion and modulation of the endocrine systems etc. Most, if not all, of these effects are thought to be mediated by AhR, a ligand-activated transcription factor. A cross-talk between AhR and androgen receptor (AR) signal transduction pathways has been observed in LNCaP and PC3/AR androgen responsive human prostate carcinoma cell lines. 2,3,7,8-Tetrachlorobenzo-*p*-dioxin (TCDD) and PAHs repress androgen-induced cell proliferation, transcription of prostate specific antigen (PSA) gene and Ah-responsive reporter genes driven by human PSA promoter or mouse mammary tumor virus (MMTV) promoter[1-4]. Testosterone has been reported to repress TCDD-induced cytochrome P450 1A1 (CYP1A1) transcription and ethoxyresorufin *O*-deethylase activities in LNCaP cells[1, 2]. While there are a number of studies on the inhibitory effects of AhR ligands on AR pathways and several mechanisms are postulated, effects of androgens on AhR pathway remain unclear, being shown with only CYP1A1 in LNCaP cells. In this study, therefore, we investigated effects of 5a-dihydroteststerone (DHT) on PAH-induced mRNA expression levels of several AhR-regulated genes in plural AR-positive cell lines to obtain further insight on gene-dependent and cell-dependent features of the inhibitory effects of androgens on AhR pathway. Genes examined in this study were CYP1A1, 1A2 and 1B1 and AhR repressor (AhRR). Cell lines used were LNCaP and human breast carcinoma T47D and MCF-7. 3-Methylcholanthrene was used as an AhR agonist.

Materials and Methods

Cell Cultures and treatment

LNCaP-FGC, T47D, and MCF-7 cells were maintained in DMEM (SIGMA, USA) supplemented with 10% fetal bovine serum (FBS). In 3-MC treatment, cells were incubated in phenol red free DMEM (SIGMA) supplemented with 5% charcoal-dextran-treated FBS and DHT of a given concentration for 24 h. Then, 3-MC was added to the medium to give desired concentration and cells were incubated for 12 h.

Real-time Reverse Transcription-PCR

Cells were incubated with 3-MC at 0.1 μM in the absence or presence of DHT at 1 μM. Total RNA was

extracted using MagExtractor (TOYOBO, Japan), and reverse-transcribed to cDNA using ExScript RT reagent Kit (TaKaRa, Japan) with oligo-dT primers. Real-time PCR was performed using the Smart CyclerII (Cepheid, USA) with Smart Cycler software (Version 2.0), and gene expression levels were determined using SYBR Premix Ex Taq (TaKaRa). The forward and reverse primers for human CYP1A1[5], 1A2[5], 1B1[6], GAPDH[6] and AhRR[7] were identical to those described previously.

Luciferase reporter assay

An Ah-responsive luciferase reporter plasmid pGL3-P-XRE3 was prepared from pGL3-promoter plasmid (Promega, USA) by inserting three tandem copies of the consensus XRE (CACGC) at the multicloning site. T47D cells were transiently transfected with pGL3-P-XRE3 plasmid and control plasmid phRL-SV40 (Promega) using Lipofectamine (Invitrogen, USA). Cells were incubated with 3-MC at 1 μM in the absence or presence of DHT of 1 or 10 μM. Luciferase activity was analyzed with whole cell lysate.

Results

Effect of DHT on mRNA levels of CYP1A1, 1A2, 1B1 and AhRR in AR positive cells

Constitutive expression of AR was observed in T47D, MCF-7 and LNCaP cells. To assess the effects of DHT on 3-MC-induced gene transcription, we measured the mRNA expression levels of CYP1A1, 1A2, 1B1, and AhRR in T47D, MCF-7 and LNCaP cells by means of real time PCR. In the absence of DHT, 3-MC significantly increased mRNA expression levels of CYP1A1, CYP1A2, CYP1B1 and AhRR in T47D and MCF-7 cells, and CYP1A1 and CYP1A2 in LNCaP cells in a concentration-dependent manner. In the presence of DHT (1 μM), mRNA levels of the target genes were elevated by 3-MC treatment, but they were significantly lower than those in the absence of DHT (Fig. 1). This result indicates that DHT inhibits the AhR-mediated up-regulation of the target genes and that the inhibitory effect of DHT is not specific to a gene or cell line. Then, the authors examined whether AR is involved in the inhibitory effect of DHT or not, by gene silencing technique with small interfering RNA (siRNA). Western blot analysis showed that treatment with siRNA for AR significantly decreased AR protein levels in the three kinds of cells. The inhibitory effect of DHT was significantly diminished by siRNA for AR, indicating that AR plays a role in the inhibitory effect of DHT.

Effect of DHT on 3-MC-induced luciferase activity in T47D cells

To address whether XRE sequence in promoter region of the target genes plays a role in the inhibitory effects of DHT or not, we prepared a plasmid pGL3-P-XRE3 in which luciferase expression is driven by three tandem copies of XRE sequence. T47D cells transiently transfected with pGL3-P-XRE3 plasmid were treated with 3-MC in the absence and the presence of DHT. 3-MC increased luciferase activity in a concentration-dependent manner. The plasmid was considered to function as a reporter vector for AhR agonists. As shown in Fig. 2, luciferase activities induced by 3-MC (1 μM) in the presence of DHT (10 μM) were significantly lower than those in the absence of DHT, indicating that DHT also inhibited the luciferase induction by 3-MC in XRE-driven reporter system.

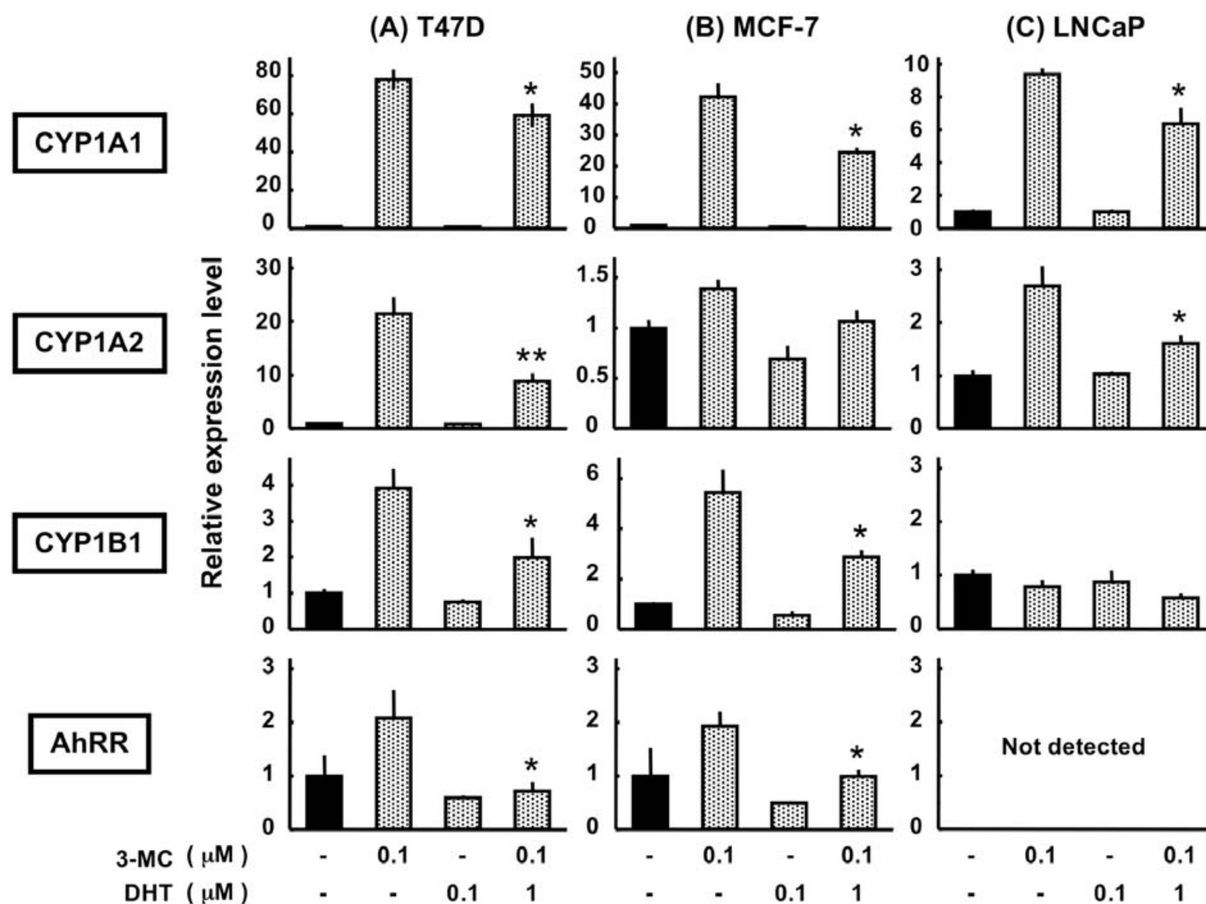

Fig. 1. Effects of DHT on 3-MC-induced mRNA levels in T47D (A), MCF-7 (B) and LNCaP cells (C). The expression levels of the target genes were corrected with expression levels of the human GAPDH as a control. Each column and vertical bar represent the mean and SD, respectively, from three or four cultures. * and **: Significantly different from corresponding 3-MC alone treatment ($p < 0.05$ and $p < 0.01$, respectively).

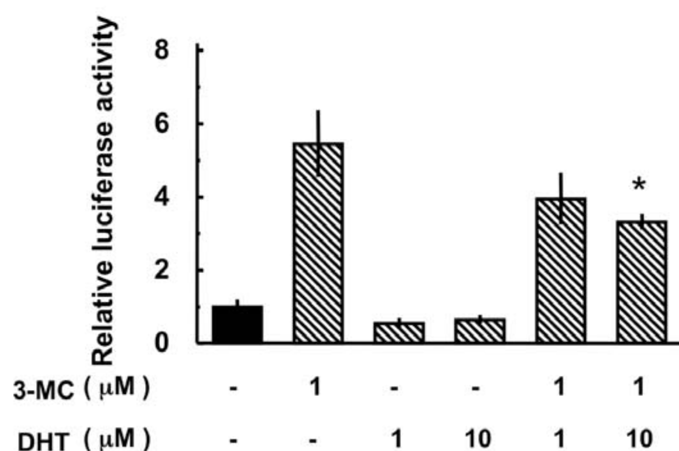

Fig. 2. Effects of DHT on 3-MC-induced luciferase activity in T47D cell. The relative luciferase activities were normalized with the *Renilla* luciferase activities as a control. Each column and vertical bar represent the mean and SD, respectively, from three cultures. *: Significantly different from corresponding 3-MC alone treatment ($p < 0.05$).

Discussion

In the present study we showed that DHT repressed 3-MC-induced gene transcription. The inhibitory effect of DHT was observed on four typical Ah-inducible genes CYP1A1, 1A2, 1B1 and AhRR in T47D and MCF-7 cells and CYP1A1 and 1A2 in LNCaP cells. As up-regulation of CYP1B1 was not induced by 3-MC and AhRR were not expressed in LNCaP cells, suppression of 3-MC-induced gene expression by DHT was observed on all genes and cell lines examined in this study, indicating that the inhibitory effect of DHT could be common to Ah-inducible genes in various kind of cell lines.

On the other hand, siRNA-based gene silencing experiment showed that knockdown of AR expression diminished the effect of DHT. Further, the effect of DHT was observed on XRE-driven luciferase reporter system. These results indicate that AR protein and XRE sequence play important roles as *trans*- and *cis*-elements, respectively. It is a possible mechanism that AR protein liganded with DHT prevent binding of AhR/Arnt heterodimer to XRE sequence present in transcription regulated regions of Ah-inducible genes by forming a protein-protein complex with AhR/Arnt.

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