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Chromatin remodeling by curcumin alters endogenous aryl hydrocarbon receptor signaling

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ABSTRACT

The aim of this study was to gain more information about the mechanisms that regulate expression of the aryl hydrocarbon receptor (AHR) target gene *CYP1A1*. Human hepatoma cells (HepG2 and Huh7) and human immortalized keratinocytes (HaCaT) were treated with different concentrations of the dietary polyphenolic compound curcumin (CUR) alone or in combination with the natural AHR agonist 6-formylindolo[3,2-*b*]carbazole (FICZ). In an earlier study, we described that CUR can activate the AHR indirectly by inhibiting metabolic clearance of FICZ. Here, we measured cell viability, activation of AHR signaling, oxidative stress and histone modifying activities in response to CUR at concentrations ranging from 0.1 to 50 μM. We observed apparent non-linear responses on cell viability and activation of AHR signaling. The *CYP1A1* expression and the CYP1A1 enzyme activity in the presence of CUR reflected the histone acetylation efficiency observed in nuclear extracts. At the lowest concentration, CUR significantly decreased histone deacetylase activity and increased the FICZ-induced CYP1A1 activity. In contrast, at the highest concentration, CUR increased the formation of reactive oxygen species, significantly inhibited histone acetylation, and temporally decreased FICZ-induced CYP1A1 activity. The results suggest that CUR can both increase and decrease the accessibility of DNA and thereby influence transcriptional responses to the ligand-activated AHR. This suggestion was supported by the fact that chromatin remodeling treatments with trichostatin A, p300, or 5-aza-dC increased *CYP1A1* transcription. We conclude that the AHR-dependent transcriptional efficiency is modified by factors that influence the cellular redox status and the chromatin structure.

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1. Introduction

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor, which belongs to the basic Per-Arnt-Sim (PAS) protein family [1]. PAS proteins are found in pathways that regulate responses to environmental changes and include, e.g., the hypoxia, and circadian response pathways as well as the AHR pathway that is activated by various endogenous and exogenous small molecules. The AHR was first named the dioxin-receptor and its functions have together with the metabolic functions of its prime targets, the cytochrome P4501 (CYP1) enzymes, been intensively investigated in the field of toxicology. However, a role for the AHR as a modulator of normal cellular signaling is emerging. Today, the receptor is known to have important functions in development, immunity as well as cancer [2–5].

A key endogenous AHR ligand is the planar and lipophilic molecule FICZ (6-formylindolo[3,2-*b*]carbazole). It is derived from tryptophan through various enzymatic as well as non-enzymatic routes and binds to the AHR with the highest affinity yet reported [6–8]. It is also an ideal substrate for the CYP1A1, 1A2 and 1B1 enzymes [9] providing a potential mechanism for control of its own steady-state levels under physiological conditions.

Several possible mechanisms that are important for the activation of *CYP1A1* transcription are currently being discussed. The classical AHR signaling pathway proceeds through binding to the

Abbreviations: AHR, aryl hydrocarbon receptor; CUR, curcumin; CYP1A1, cytochrome P4501A1; FICZ, 6-formylindolo[3,2-*b*]carbazole; HAT, histone acetyltransferase; HDAC, histone deacetylase; TSA, trichostatin A; 5-Aza-dC, 5-Aza-2′-deoxycytidine.

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receptor of an endogenous or exogenous ligand, leading to dimerization with the AHR partner protein ARNT, and formation of a DNA-binding AHR complex (reviewed in Ref. [10]). Examples of exogenous activators are dioxins and other dioxin-like halogenated aromatic compounds. However, many compounds with no or very low affinity for binding to the AHR can also activate the AHR signaling [11]. To understand such non-classical AHR-activation, several explanations have been put forward including the claim that the receptor is highly promiscuous [10]. We have begun to elucidate an indirect mechanism for AHR activation by which different agents can activate *CYP1A1* expression by inhibiting the metabolic breakdown of FICZ [9,12–15].

We have noted that agents such as H_2O_2, UVB, and the sulfhydryl (SH) reactive metals As^{3+}, Cd^{2+}, Hg^{2+}, and Ni^{2+}, which increase the cellular oxidation state, lead to a temporal blockage of *CYP1A1* transcription [9,12–15]. The mechanism for this blockage was clarified in human cell lines treated with As^{3+}. We found that an initial activation of NADPH oxidases, mediated an oxidative stress triggered NFE2-related transcription factor 2 (Nrf2) dependent antioxidant defenses, which resulted in a shift towards more reducing conditions. Under these conditions, characterized by regained levels of reduced glutathione (GSH), the cells were highly sensitive to activation by low levels of FICZ [14]. From these experiments we have concluded that the redox potential of the cell strongly influences the AHR-mediated *CYP1A1* transcription process by increasing the response to ligands. In this respect, what has many times been reported as ligand-independent activation of AHR may well be explained by increased responsiveness to background levels of FICZ in cell culture media [16]. Also in the previous study of the polyphenols curcumin (CUR), resveratrol (RES) and quercetin (QUE), we suggested that the induction mechanism was indirect [13]. All three compounds inhibited the metabolic turnover of FICZ and activated AHR signaling only in medium that contained trace amounts of FICZ.

It is known that the bioactive compound CUR can influence the cellular redox potential and behave both as an antioxidant and as a pro-oxidant [17]. CUR is also a known to affect histone acetylation processes and has been described to activate or inactivate gene expression through histone modification, DNA methylation, and miRNAs at micro molar concentrations [18,19]. In our previous study, a low concentration of CUR stimulated FICZ-mediated *CYP1A1* expression while at higher concentrations CUR caused the temporal transcription inhibition, as described above, after which the *CYP1A1* expression was increased. Therefore, in the present study, we set out to investigate whether changes of the cellular redox state and chromatin remodeling was involved in the nonlinear dose-responses in AHR activation by CUR.

2. Materials and methods

2.1. Chemicals and kits

Chemicals were obtained from the following suppliers: FICZ from Syntastic AB, Sweden; 5-aza-2′-deoxycytidine (5-Aza-dC), buthionine-(S,R)-sulfoximine (BSO), 2′,7′-dichlorofluorescein diacetate (DCFH-DA), 3,4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), 5, 5′-dithiobis-(2-nitrobenzoic acid (DTNB), 7-ethoxyresorufin (EOR), N-acetyl-L-cysteine (NAC), and trichostatin A (TSA) from Sigma-Aldrich, Germany; lysis buffer from Promega, Madison, WI; DC protein assay kit from Bio Rad, Sweden; D-luciferin from BioTherma, Sweden; DharmacoFECT reagent from Dharmacon, Lafayette, CO; NE-PER nuclear extraction reagents kit (no. 78833) from Thermo Scientific; histone acetyltransferase (HAT) activity assay kit (no. EPI001) and histone deacetylase (HDAC) fluorometric assay kit (no. CS1010) from Sigma-Aldrich, Germany. All cell culture reagents and media were purchased from Invitrogen, Carlsbad, CA.

2.2. Cell culture and chemical treatments

Three human hepatoma cell lines (HepG2, HepG2-XRE-Luc and Huh7) and the immortalized human keratinocytes (HaCaT) were used in the experiments. The HepG2-XRE-Luc cell line containing a pTX.DIR-luciferase reporter under the control of a model *CYP1A1* promoter consisting of two XRE sequences of the rat *CYP1A1* gene [20] and the herpes simplex virus thymidine kinase promoter was kindly provided by K. Gradin, Karolinska Institutet. We showed in an earlier study that the endogenous human CYP1A1 mRNA response to FICZ is identical in these cells to that seen when luciferase activity is used as an endpoint [12]. The immortalized human keratinocyte cell line HaCaT was kindly provided by N. E. Fusenig (DKFZ, Heidelberg, Germany). All cells were grown in DMEM supplemented with 10% fetal bovine serum (FBS), 100 μg/mL streptomycin, and 100 IU/mL penicillin under an atmosphere containing 5% CO_2 at 37 °C. The XRE-HepG2 medium was additionally supplemented with 800 μg/mL geneticin. Treatments were started by replacing the growth medium with fresh medium without FBS containing different concentrations of the test compounds. The lengths of treatments varied and are indicated in the results section. 5-Aza-dC was added to HepG2 cells during a pretreatment period of 72 h.

2.3. MTT analyses of cell viability

To determine the effects of CUR on cell viability, the cells were plated into 96-well plates and grown for 24 h and then treated with different concentrations of CUR for another 24 h. After the incubation period, the treatments were terminated by removing the medium and rinsing the cells with PBS. The cells were exposed to the MTT dye for 4 h and the blue purple formazan crystal products were dissolved in DMSO for quantification by measuring the absorption at 570 nm.

2.4. Analyses of CYP1A1 enzyme activity

Whole cell EROD activities were measured to estimate CYP1A1 activity. Cells were seeded at high density in 96-well plates and grown to over-confluence. At the indicated time points, the medium was removed and the cells were rinsed with PBS. The EROD reaction was initiated by addition of 2 μM EOR in sodium phosphate buffer (50 mM pH 8.0). After 20 min of incubation at 37 °C, the formation of resorufin was measured. Fluorescence was read at excitation and emission wavelengths of 535 nm and 590 nm. Data were normalized to cellular protein content determined with the DC protein assay kit.

2.5. Analyses of CYP1A1 reporter activity

Transcription of the *CYP1A1* gene was estimated using a luciferase reporter assay in HepG2-XRE-Luc cells. Cells were grown in 24-well plates and treated when they had reached 80% confluence. Five hours after the start of treatment, the medium was removed, and the cells were briefly washed with ice-cold PBS and harvested in 50 μL cell culture lysis buffer. Luciferase activity was measured in 30 μL cell extracts after the addition of 40 μL of ATP and 40 μL of D-luciferin. Luciferase activity was normalized to cellular protein content determined with the DC protein assay kit.

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