Down-Regulation of Aryl Hydrocarbon Receptor–Regulated Genes by Tumor Necrosis Factor-α and Lipopolysaccharide in Murine Hepatoma Hepa 1c1c7 Cells

NEGAR GHARAVI, AYMAN O.S. EL-KADI

Faculty of Pharmacy and Pharmaceutical Sciences, 3118 Dentistry/Pharmacy Centre, University of Alberta, Edmonton, AB, Canada T6G 2N8

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ABSTRACT: Although much is known concerning the effects of inflammation and oxidative stress on the cytochrome P450 1A1 (CYP1A1), little is known about the modulation of other aryl hydrocarbon receptor (AHR)-regulated genes such as glutathione-*S*-transferase Ya (GST Ya) and NAD(P)H:quinone oxidoreductase (QOR) by inflammation. In the present study, the effect of tumor necrosis factor (TNF)-α and lipopolysaccharides (LPS) on the constitutive and inducible expression of the AHR-regulated genes cyp1a1, GST Ya, and QOR was determined in murine hepatoma Hepa 1c1c7 (WT), AHR-deficient (C12), and AHR nuclear translocator protein (ARNT)-deficient (C4) cells. We found that both TNF-α and LPS strongly repressed the constitutive expression and the β-naphthoflavone-mediated induction of cyp1a1, GST Ya, and QOR in WT but not in C12 and C4 cells. The induction of GST Ya and QOR activities and mRNA levels by phenolic antioxidant, *tert*-butylhydroquinone, through the antioxidant response element was not significantly affected by TNF-α or LPS. In addition, a significant increase in reactive oxygen species was observed in WT, C12, and C4 cells treated with TNF-α or LPS which was completely prevented by *tert*-butylhydroquinone. These results show that the down-regulation of AHR-regulated genes by TNF-α and LPS is dependent on the presence of both heterodimeric transcription factors, AHR and ARNT. Furthermore, reactive oxygen species may be involved in the down-regulation of AHR-regulated genes. J Pharm Sci 94:493–506, 2005

Keywords: cell culture; hepatocytes; Phase I enzyme; Phase II enzymes; cytochrome P450; glutathione-*S*-transferases (GST); aryl hydrocarbon receptor; inflammation; lipopolysaccharide; NAD(P)H:quinone oxidoreductase; tumor necrosis factor-α

INTRODUCTION

The aryl hydrocarbon receptor (AHR) is a ligand-activated basic helix-loop-helix transcription factor that controls the expression of a host of different genes whose functions are linked to the metabolism of dietary constituents, drugs, and potentially hazardous agents such as environmental contaminants.[1,2] The AHR exists as cytoplasmic aggregates bound to two 90-kDa heat-shock proteins, the cochaperone p23 and the 43-kDa protein termed hepatitis B virus X-associated protein.[3–5] Upon ligand binding, the AHR dissociates from 90-kDa heat-shock proteins and the ligand-receptor complex translocates to the nucleus. Then, the activated AHR dimerizes with the AHR nuclear translocator protein (ARNT), and binds to a class of promoter DNA sequences called xenobiotic responsive element of the target gene to activate their transcription.[6,7]

Correspondence to: Ayman O.S. El-Kadi (Telephone: 780-492-3071; Fax: 780-492-1217;
E-mail: aelkadi@pharmacy.ualberta.ca)

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The AHR-regulated genes consist of four Phase I enzymes, cytochrome P450 1A1 (CYP1A1), CYP1A2, CYP1B1, and CYP2S1 and four Phase II xenobiotic metabolizing enzymes, including NAD(P)H: quinone oxidoreductase (QOR), glutathione-*S*-transferase Ya subunit (GST Ya), cytosolic aldehyde dehydrogenase-3, and UDP-glucuronosyltransferase 1A6.[8,9] These enzymes are abundant in tissues that have an important role in first-pass metabolism, digestion, and drug metabolism, such as those of the gastrointestinal tract and the liver. For the most part, these enzymes could be considered to have an important role in drug metabolism. However, the induction of certain heme-thiolate proteins, such as CYP1A1, is considered to be potentially counterproductive to this process because CYP1A1 is capable of producing epoxides and dihydrodiol epoxides from aromatic and halogenated hydrocarbons.[10] These metabolites have been shown to be involved in the mediation of a broad range of distinct toxic responses such as immune suppression, endocrine disruption, birth defects, and carcinogenesis.[11] The mechanism for these AHR-mediated pathophysiological conditions is not well understood.

In animals, lipopolysaccharide (LPS) or turpentine administration has been shown to down-regulate CYP1A1 and CYP1A2.[12,13] In humans, LPS increased serum concentrations of tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6 and, in turn, decreased the clearance of antipyrine, hexobarbital, and theophylline.[14] *In vitro*, IL-6 repressed CYP1A1, CYP1A2, and CYP3A mRNA in human hepatoma cells.[15] IL-1β, IL-6, and TNF-α inhibited CYP1A2, CYP2D, CYP2E1, and CYP3A mRNA and related enzyme activities in human primary hepatocytes.[16] Furthermore, inhibition of polycyclic aromatic hydrocarbon–mediated induction of CYP1A expression by cytokines and growth factors has also been demonstrated in different cell systems *in vitro*. For example, polycyclic aromatic hydrocarbon induction of CYP1A mRNA expression is inhibited by IL-1β, IL-6, and TNF-α.[17–19]

Although much is known concerning the effects of inflammation on the CYP1A1, very little is known about the other AHR-regulated genes such as GST Ya and QOR. However, it is well known that GST Ya and QOR are highly inducible by AHR agonists such as β-naphthoflavone (βNF) or by phenolic antioxidants such as *tert*-butylhydroquinone (tBHQ).[20] βNF is also termed bifunctional inducers, which induces both Phase I and II enzymes through AHR- and antioxidant response element (ARE)-dependent mechanisms. However, tBHQ and similar inducers are designated as monofunctional inducers, which induce only Phase II enzymes, including GST Ya and QOR, by activation of ARE and through AHR-independent mechanisms.[21,22] Because the levels of Phase I and II drug metabolizing enzymes in an organism may determine the potential for either metabolic activation of drugs or decreased efficacy of therapeutic molecules during inflammation, it is important to study the mechanism involved in the modulation of these enzymes during inflammation and oxidative stress. The major aim of this study was to investigate the effect of the proinflammatory cytokine, TNF-α, and LPS on the basal and inducible expression of the AHR-regulated genes cyp1a1, GST Ya, and QOR in the Hepa 1c1c7 cells.

EXPERIMENTAL

TNF-α was obtained from Peprotech Canada (Ottawa, ON). LPS, βNF, nicotinamide adenine dinucleotide phosphate, cumene hydroperoxide, dicoumarol, 2,6-dichlorophenolindophenol, flavin adenine dinucleotide, glutathione reductase, reduced glutathione, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 7-ethoxyresorufin, 7-methoxyresorufin, bovine serum albumin (BSA), Dulbecco's modified Eagle medium (DMEM) base, fluorescamine, glucose, protease inhibitor cocktail, anti-goat immunoglobulin G peroxidase secondary antibody, and tBHQ were purchased from Sigma-Aldrich (St. Louis, MO). Tris hydrochloride, agarose, and sodium azide were purchased from EM Science (Gibbstown, NJ). Tween-20 was from BDH Inc. (Toronto, ON). Amphotericin B, resorufin, 100X vitamin supplement solution, and amphotericin B were purchased from ICN Biochemicals Canada (Toronto, ON). Hybond-N-nylon filters were from Amersham Canada (Oakville, ON). Penicillin/streptomycin, gentamycin, L-glutamine, fetal bovine serum, nonessential amino acid solution, TRIzol reagent, and the random primers DNA labeling system were obtained from Invitrogen Canada (Burlington, ON). [α-^{32}P]dCTP (3000 Ci/mmol) was supplied by PerkinElmer (Boston, MA). Bromophenol blue, β-mercaptoethanol, glycine, acrylamide, *N*′,*N*′-bis-methylene-acrylamide, ammonium persulphate, nitrocellulose membrane (0.45 μm), and TEMED were purchased from Bio-Rad Laboratories (Hercules, CA). CYP1A1 goat anti-mouse polyclonal primary antibody (G-18) was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz,