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Dexamethasone induces human glutathione S transferase alpha 1 (hGSTA1) expression through the activation of glucocorticoid receptor (hGR)

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ABSTRACT

The glutathione S transferases (GSTs) are a superfamily of isoenzymes that play an important role in xenobiotic and endobiotic detoxification, cellular protection from oxidative stress and modulation of signalling pathways. Human GSTA1 (hGSTA1), a cytosolic isoform, is the most abundant GST in the liver and is involved in the metabolism of carcinogenic compounds, chemotherapeutic agents and lipid peroxidation products. However, the molecular mechanism underlying the regulation of hGSTA1 expression is not well understood. Therefore, the aim of the present study was to better understand the regulation of *hGSTA1* gene expression.

Putative response elements for several nuclear receptors, including the glucocorticoid receptor (GR), have been identified at the *hGSTA1* gene promoter located at −896 bp, −863 bp and −727 bp from the transcription start site. After dexamethasone (DEX) treatment, the GR induces hGSTA1 expression at the transcriptional level. The characterisation of this effect reveals that the GR binds to several glucocorticoid response elements (GREs) located at the *hGSTA1* gene promoter. This interaction also results in the transactivation of the *hGSTA1* gene promoter together with an increase of the hGSTA1 mRNA levels as well as the protein and activity levels in HepG2 cells. Together, the present results suggest that glucocorticoids have the potential to alter the xenobiotic and endobiotic metabolism mediated by hGSTA1.

1. Introduction

Glutathione S-transferases (GSTs) are an enzyme superfamily critical for detoxification processes in animals, plants and microorganisms. GSTs activate the sulphur atom of glutathione and catalyse the conjugation of reduced glutathione (GSH) with hydrophobic, non-polar substrates possessing an electrophilic centre (Hayes et al., 2005; Prabhu et al., 2004). Conjugation with GSH renders substrates more soluble for cellular export proteins, such as the multidrug resistance-related protein (Suzuki et al., 2001). The range of compounds detoxified by GSTs is remarkably diverse and includes carcinogens, anticancer drugs, and environmental toxicants (Hayes et al., 2005; Prabhu et al., 2004). In addition, functions not related to detoxification have been uncovered for GSTs, including signal transduction, steroid biosynthesis and intracellular transport (Fedulova et al., 2011).

Three major families of proteins that are widely distributed in nature exhibit GST activity. Two of these, the cytosolic and mitochondrial GSTs, comprise soluble enzymes that are only distantly related. The third family comprises microsomal GSTs and is now referred to as membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG) (Oakley, 2011). Cytosolic GSTs are present as homodimers or heterodimers of subunits ranging from 24.5 to 28.5 kDa and are further classified into seven groups: alpha, kappa, mu, pi, theta, zeta, omega and sigma.

Originally termed "ligandin", the human GST (hGST) alpha class, consists of four distinct proteins: hGSTA1–hGSTA4. hGSTA1 is the most abundant GST in the liver and catalyses glutathione conjugation of a wide range of electrophiles. It is the principal GST responsible for the detoxification of xenobiotics and is involved in the metabolism of carcinogenic compounds, chemotherapeutic agents and lipid peroxidation products. hGSTA1 also possesses glutathione-dependent steroid isomerase activity and glutathione-dependent (selenium-independent) peroxidase activity (Coles and Kadlubar, 2005; Koumaravelou et al., 2011).

Although hGSTA1 plays an important role in detoxifying a wide spectrum of electrophilic compounds and has been implicated in a variety of resistance phenomena involving cancer chemotherapeutic agents, insecticides, herbicides and microbial antibiotics (Sheehan et al., 2001), the molecular mechanism underlying the regulation of hGSTA1 expression is not well understood. In rodents, it has been reported that cytosolic GSTA1 is up-regulated by pregnenolone (Maglich et al., 2002), 3-methyl-cholantrene, beta naphthoflavone

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(Lindros et al., 1998), 1,4-bis-[2-(3,5-dichloropyridyloxy)]benzene,3,3′,5,5′-tetrachloro-1,4-bis(pyridyloxy)benzene (TCPOBOP) (Aleksunes and Klaassen, 2012) and oltipraz, suggesting that pregnenolone X receptor (PXR), aryl hydrocarbon receptor (AHR), constitutive androstane receptor (CAR) and the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) might be implicated in the regulation of GSTA1 expression. The information available on hGSTA1 is even more limited. Previous studies have shown that PXR, AHR and Nrf2 ligands increase hGSTA1/2 mRNA levels (Morel et al., 1993), suggesting a role for these transcription factors in *hGSTA1* and/or *hGSTA2* gene expression. However, these studies do not distinguish between the two isoforms.

Therefore, the aim of the present study was to identify the ligand-dependent transcription factors involved in the control of *hGSTA1* gene expression and to characterise the molecular mechanism involved in such regulation.

2. Materials and methods

2.1. Materials

HepG2 cells were obtained from the American Type Culture Collection (Manassas, VA, USA). Phenylmethanesulfonyl fluoride (PMSF), dexamethasone (DEX), dimethyl sulfoxide (DMSO) and actinomycin-D (Act-D) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. In silico analysis

The *hGSTA1* gene promoter was analysed using the Transcriptional Factor Search (http://www.cbrc.jp/research/db/TFSEARCH.html) and ALGGEN PROMO (http://alggen.lsi.upc.es/cgibin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3) databases to determine the potential response elements for type 1 and type 2 nuclear receptors. Scores greater than 80 were included in the analysis.

2.3. Cell culture

HepG2 cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Camarillo, CA, USA) supplemented with 10% foetal bovine serum (Hyclone, Logan, UT, USA), penicillin (100 μg/mL), streptomycin (100 μg/mL), 1% L-glutamine and 1% non-essential amino acids (Invitrogen, Camarillo, CA, USA). Cell cultures were maintained in 75-cm^2 flasks at 37 °C in a humidified incubator with a 5% CO_2 atmosphere.

2.4. Viability test

Cell viability was determined using the MTT test assay. Briefly, 20 μl of MTT (5 mg/mL in PBS) were added to each well and incubated for 4 h; 100 μl of 10% SDS in 0.01 N HCl were added to dissolve MTT residues, and the optical density was determined on an ELISA-plate reader (Bio-Rad Laboratories, Hercules, CA) at 590 nm.

2.5. Real-time quantitative reverse transcription–polymerase chain reaction (RT-PCR) analysis

Total RNA was prepared from cultured HepG2 cells using the TRIzol reagent, according to the manufacturer's instructions (Invitrogen, Camarillo, CA, USA). RNA was quantified spectrophotometrically at 260 nm, and the purity was assessed by the ratio of the optical density at 260 nm to that at 280 nm. The RNA integrity was evaluated by electrophoresis on 1% agarose gels. cDNA was prepared for the quantitative PCR from 2 μg of total RNA using SuperScript® II Reverse Transcriptase (Invitrogen, Camarillo, CA, USA) and oligo dT. PCRs were performed in a StepOne Real-Time PCR System (Applied Biosystems, Branchburg, NJ, USA) and analysed using the comparative threshold cycle method. The mRNAs encoding *TAT*, *hGSTA1* and *GAPDH* were amplified in a single PCR to allow for normalisation of the mRNA data. The PCR mixture contained 2 μL of cDNA, 1× TaqMan Universal PCR Master Mix (Applied Biosystems, Branchburg, NJ, USA), 0.9 μM primers and 0.25 μM probes. The primer and probe sequences used for *hGSTA1* were as follows: 5′-GGACGGTGACAGCGTTTAAC-3′ (forward), 5′-GGCTTCTCTGCCATGATAGCA-3′ (reverse); and probe (FAM): AAAGCTTAGAGAAACCTCC. The probes used for *TAT* and *GAPDH* mRNAs were obtained from Applied Biosystems (Branchburg, NJ, USA), with identification numbers Hs00356930_m1_F and 4326317E, respectively.

2.6. Plasmids

The reporter plasmid pGL4/-1593*hGSTA1*, including the *hGSTA1* gene promoter, was constructed as follows. A −1593/ + 1 fragment was generated by PCR amplification using human genomic DNA and oligonucleotides (forward: 5′- CAGATTTCCAAACTCCCCATA-3′, reverse: 5′- ATATTTATGTAATCCTCCTGGACCTTAAG-3′). The fragment was then cloned into the pCR 2.1-TOPO transition vector (Invitrogen, Camrillo, CA, USA). To verify the amplicon orientation as well as its correct sequence, single and double restriction enzyme digestions and sequencing were performed. Then, the fragment was cloned into the KpnI/Xho1 site of the pGL4.10 [luc2] reporter vector (Promega, Madison, WI, USA) containing the firefly luciferase gene; this plasmid was designated as pGL4/-1593*hGSTA1*. The reporter plasmids pGL4/-1593*hGSTA1*-GRE1mut, pGL4/-1593*hGSTA1*-GRE2mut, and pGL4/-1593*hGSTA1*-GRE3mut were produced by PCR-based site-directed mutagenesis according to the standard QuikChange II Site-Directed Mutagenesis Kit (Agilent technologies, Sta. Clara. CA. USA) using the pGL4/-1593*hGSTA1* reporter vector as a template. Mutagenic primers were designed using the web-based QuikChange Primer Design Program available online at www.agilent.com/genomics/qcpd and the nucleotide targets were selected based on the most conserved positions (GRE1mut Forward: 5′-CCAGCACCTCACCCCCT ACGTGTTGTGTCTTCTCTT-3′, Reverse 5′-AAGAGAAGACACAACAC GTAGGGGGTGAGGTGCTGG-3′; GRE2mut Forward 5-GTGTCTTCTC TTCTCTCTGCTGTGAAAACAATTGTCATTGGATTTGG-3′, Reverse: 5′-CCAAATCCAATGACAATTGTTTTCACAGCAGAGAGAAGAGAAGACAC-3′; GRE3mut Forward: 5′-GATCTAGGGATTTCTATATGAAC TTATCTTTCTTGGGGGCCAAC-3′, Reverse: 5′-GTTGGCCCCCAAGA AAGATAAGTTCATATAGAAATCCCTAGATC-3′).

The pCMV-hGR expression vector was donated by Dr. John A. Cidlowski (National Institute of Environmental Health Sciences, USA).

2.7. Transient transfection

HepG2 cells were cultured in DMEM as described above. Transfection was performed using an Eppendorf Multiporator (Eppendorf, Westbury, NY, USA). Each sample (1×10^6 cells/mL) was resuspended in 400 μL of hypo-osmolar buffer (Eppendorf, Westbury, NY, USA) containing 5 μg of pGL4/-1593*hGSTA1* and 1 μg of pRL-CMV as an internal control. Co-transfected cells contained 5 μg of pGL4/-1593*hGSTA1* and 1 μg of hGR expression vector pCMV-hGRα.

A single 450-V, 100-μs pulse was delivered to each sample. After electroporation, the transfected cells were seeded in 6-well plates with 2 mL of DMEM supplemented with 5 mM sodium butyrate and cultured for 12 h. The medium was replaced with fresh medium, and the cells were treated with vehicle (DMSO) or 0.1 μM DEX. After 24 h, the cells were homogenised for the enzymatic assay by incubation with passive lysis buffer (Promega, Madison, WI, USA) for 15 min at room temperature. Luciferase activity was determined using the Dual-Glo Luciferase Reporter Assay System (Promega, Madison, WI, USA), according to the manufacturer's instructions, in a Modulus luminometer (Turner Biosystems, Sunnyvale, CA, USA). Blanks were obtained by performing luciferase activity assays in mock-transfected cells. The firefly luciferase