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Transfection of HepG2 cells with *hGSTA4* provides protection against 4-hydroxynonenal-mediated oxidative injury

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Abstract

4-hydroxynonenal (4-HNE) is a mutagenic α,β -unsaturated aldehyde produced during oxidative injury that is conjugated by several glutathione *S*-transferase (GST) isoforms. The alpha class human GSTA4-4 enzyme (hGSTA4-4) has a particularly high catalytic efficiency toward 4-HNE conjugation. However, hGST4-4 expression is low in most human cells and there are other aldehyde metabolizing enzymes that detoxify 4HNE. In the current study, we determined the effect of over-expression of *hGSTA4* mRNA on the sensitivity of HepG2 cells to 4-HNE injury. HepG2 cells transfected with an *hGSTA4* vector construct exhibited high steady-state *hGSTA4* mRNA, high GST-4HNE catalytic activities, but lower basal glutathione (GSH) concentrations relative to insert-free vector (control) cells. Exposure to 4-HNE elicited an increase in GSH concentrations in the control and hGSTA4 cells, although the dose-response of GSH induction differed among the two cell types. Specifically, hGSTA4 cells had significantly higher GSH concentrations when exposed to 5–15 μ M 4-HNE, but not at 20 μ M 4-HNE, suggesting extensive GSH utilization at high concentrations of 4-HNE. The hGSTA4 cells exhibited a significant growth advantage relative to control cells in the absence of 4-HNE, and a trend towards increased growth at low dose exposures to 4-HNE. However, the hGSTA4 cells did not exhibit a growth advantage relative to control cells at higher 4-HNE exposures associated with increased GSH utilization. As expected, the hGSTA4 cells showed resistance to 4-HNE-stimulated lipid peroxidation at all 4-HNE doses. In summary, our data indicates that over-expression of hGSTA4 at levels conferring high GST-4-HNE conjugating activity confers a partial growth advantage to HepG2 cells and protects against 4-HNE oxidative injury. However, the loss of proliferative capacity of hGSTA4 cells challenged with levels of 4-HNE associated with severe oxidative stress indicates a role of other aldehyde metabolizing enzymes, and/or GSH-electrophile transporter proteins, in providing full cellular protection against 4-HNE toxicity.

Keywords

glutathione *S*-transferase; hGSTA4; 4-hydroxynonenal; lipid peroxidation

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1. Introduction

A number of breakdown products are released under conditions of cellular oxidative stress that decompose to produce high concentrations of highly reactive α,β -unsaturated aldehydes. In particular, 4-hydroxynonenal (4-HNE) is a mutagenic aldehyde released during lipid peroxidation which readily forms covalent adducts with DNA and proteins (Esterbauer *et al.*, 1991). Physiological conditions that elevate cellular 4-HNE can result in a loss of mitochondrial function and increased cellular necrosis leading to a loss of overall cell viability (Ramachandran *et al.*, 2001). Coincidentally, elevated tissue 4-HNE concentrations have been associated with several human diseases including cancer, Parkinson's disease, Alzheimer's disease, atherosclerosis, pulmonary inflammation, rheumatoid arthritis and ophthalmologic disorders (reviewed in Esterbauer *et al.*, 1991). In addition to its cellular toxicity, 4-HNE plays an important role in cell signaling pathways and can mediate cell cycle arrest (Yang *et al.*, 2003), apoptosis (Raza and John, 2006), and stimulate cell proliferation (Moneypenny and Gallagher, 2005).

Given the high reactivity and physiological relevance of 4HNE, a number of enzyme systems have evolved to regulate 4-HNE levels in different cells and tissues. The primary enzymatic pathways of 4-HNE detoxification in liver include aldehyde dehydrogenase (ALDH), alcohol dehydrogenase (ADH), aldehyde reductase (ALRD), and GST (Canuto *et al.*, 1993; Luckey and Petersen, 2001, Gallagher and Gardner, 2002; Gardner *et al.*, 2003). Of these enzyme systems, GST is a predominate mechanism for protection against 4-HNE toxicity in mammalian liver (Hartley and Petersen, 1997; Gardner *et al.*, 2003). Interestingly, the major human liver GST isoform, hGSTA1-1, is relatively inefficient at conjugating 4-HNE ($K_m = 50$ mM, $k_{cat}/K_m = 0.058$ s⁻¹mM⁻¹ (Hubatsch *et al.*, 1998; Coles and Kadlubar, 2005) and other alkenal substrates. However, another alpha class isoform, hGSTA4-4, appears to be the most efficient human GST isoform that mediates 4-HNE conjugation with a K_m of 49 μ M and a rapid k_{cat}/K_m of 2.7 s⁻¹mM⁻¹ (Cheng *et al.*, 2001). Despite its high catalytic efficiency toward 4-HNE, the contribution of this GST isoform toward 4-HNE removal among competing enzymatic pathways is unknown due to its relatively low expression in human tissues (Gallagher and Gardner, 2002).

Over-expression of an alpha class GST in mice with high 4-HNE conjugating activity (mGSTA4-4) provides cellular protection against the cytotoxic effects of oxidative stress mediated by hydrogen peroxide and organic hydroperoxides (Zimniak *et al.*, 1997) and protects against 4-HNE induced apoptosis by inhibition of JNK-mediated cell signaling (Cheng *et al.*, 2001). In addition, incorporation of hGSTA4-4 into adherent cell lines can cause immortalization from changes in 4-HNE mediated signaling events (Sharma *et al.*, 2004). However, what is not known is if cells expressing high levels of hGSTA4-4 are resistant to 4-HNE-mediated oxidative damage relative to cells expressing low, but normal physiological hGSTA4-4 levels. In the current study, we tested the hypothesis that stable transfection of *hGSTA4* mRNA would result in an increased cellular capacity to detoxify 4-HNE and confer protection against 4HNE-mediated oxidative damage. Human HepG2 cells were selected for study due to their low constitutive expression of the hGSTA4-4 protein (Zimniak *et al.*, 1997).

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

All custom designed primers, platinum *Taq* polymerase, T4 DNA ligase, penicillin, streptomycin, fetal bovine serum (FBS), Trizol reagent and the pcDNA3.1 mammalian expression vector were purchased from Invitrogen Inc. (Carlsbad, CA). BamHI and HindIII were purchased from New England Biolabs (Ipswich, MA) and the human HepG2 cells were

purchased from American Type Culture Collection (Manassas, VA). Agarose was obtained from Bio-Rad Laboratories (Hercules, CA), and the pGEM-TZ cloning vector was purchased from Promega Corp (Madison, WI). Earle's MEM media was purchased from Gibco-BRL (Gaithersburg, MD). Trypan blue, dimethyl sulfoxide (DMSO), and other biochemicals were purchased from Sigma Chemical Company (St. Louis, MO). Racemic 4-HNE was purchased from Cayman Chemical (Ann Arbor, MI) and Alamar blue was purchased from Bio-Source International (Camarillo, CA).

2.2 Cell culture and transfection

Human hepatoma HepG2 cells were grown in Earle's MEM medium containing 10% heat inactivated fetal calf serum, 100 µg streptomycin/ml, 100 units penicillin/ml in the presence of 5% CO₂ (Zimniak *et al.*, 1997). The *hGSTA4* cDNA (Gardner and Gallagher, 2001) was excised from pGEM-TZ plasmid and the cDNA insert was blunt-end ligated into the pcDNA3.1 mammalian expression vector. Initially, the *hGSTA4* construct lacked a functional Kozak initiator sequence and contained splice sites that caused mRNA splicing *in vitro*. Furthermore, a point mutation that resulted in an unwanted proline→leucine mutation was found in the recombinant cDNA. The native 5' untranslated region which contains a Kozak sequence was added and a silent point mutation to eliminate the donor splice site, and the proline→leucine mutation was removed. HepG2 cells were then transfected with the modified pcDNA3.1/*hGSTA4* plasmid by calcium phosphate co-precipitation (Watanabe *et al.*, 1997) and several cell lines were selected in geneticin. Other HepG2 cells were transfected with the insert-free pcDNA3.1 vector (hereby termed "control cells").

2.3 Biochemical and molecular characterization of transfected cells

HepG2 cell lysates were sonicated in 0.1M KH₂PO₄ (pH 7.4) and centrifuged at 10,000 × *g* and immediately stored at −80°C. Total cellular RNA was prepared separately using the Trizol reagent (Doi *et al.*, 2004). GST activity in cell supernatants was determined using 1-chloro-2,4-dinitrobenzene (CDNB) and 4-HNE as substrates (Gallagher and Gardner, 2002). GSH peroxidase activity in cell lysates was determined using cumene hydroperoxide (CumOOH) and hydrogen peroxide as substrates (Hughes and Gallagher, 2004). Protein concentrations in cell lysates were determined by the bicinchoninic method with bovine serum albumin as the standard. Total GSH concentrations were determined in acidified 10,000 × *g* supernatants of HepG2 cells by the method of Baker *et al.* (Baker *et al.*, 1990) using a 96-well microplate reader (Spectramax 250, Molecular Devices, Sunnyvale CA). Standard curve values were obtained from serial dilutions of GSH standards.

Analysis of steady state *hGSTA4* mRNA expression was performed using semi-quantitative multiplexed RT-PCR and *hGSTA4*-specific PCR primers (forward primer 5'-AGTTGCAGGATGGTAACCACC-3', reverse primer 5'-ATGGCCTAAAGATGTTGTAGACGG 3') and including the housekeeping gene β-globin (forward primer 5'-GAAGAGCCAGGACAGGTACGG-3', reverse primer 5'-ACCAACTTCATCCACGTTTCACC-3'). Each PCR reaction included total first strand cDNA from HepG2 cells, 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM of each dNTP, and 100 ng of β-globin and GST primers. After a 5 min incubation at room temperature, *Taq* DNA polymerase was added to the reactions. PCR was carried for 30 cycles of 30 s at 94°C, 30 s at 56°C, 30 s at 72°C; and 5 min at 72°C. PCR products were separated on a 2% agarose gel and quantitated using a Bio-Rad Fluor-S imaging system. The conditions were chosen so that none of the RNAs analyzed reached a plateau at the end of the amplification protocol (*i.e.* in the exponential phase of amplification) and also so that the two sets of primers used in each reaction were not competitive. Each set of reactions included a no-sample negative control. In addition, negative controls containing RNA in lieu of cDNA were included to exclude genomic DNA contamination in the reactions.

2.4 Effect of over-expression of hGSTA4 on cell growth, GSH concentrations and lipid peroxidation by 4HNE

For initial 4-HNE dose-response experiments, HepG2 cells were plated in 96 well plates and cultured according to Zimniak *et al.* (Zimniak *et al.*, 1997). Cell culture media was supplemented with ethanol vehicle control (0.05% v/v), 5, 10, 15 or 20 μ M 4-HNE without a media change prior to addition of 10% Alamar blue. At 1, 2, 4, 8 and 24 hr, the cells were analyzed for the effects of 4-HNE on cell growth by Alamar blue reduction at 554 nm excitation/590 nm emission (Ahmed, 1994) using a 96-well fluorescence microplate reader. To analyze treatment-related effects on relative cell growth, the initial Alamar blue fluorescence intensity measurements taken at 1 hr post-addition of test agent (or vehicle) were subtracted from values obtained at 2, 4, 8 and 24 hrs post-dosing. Three replicates were carried out and analyzed statistically as described below in section 2.5.

After establishing a dose-response and time course of 4-HNE effects on cell growth, subsequent oxidative damage experiments involved incubation of HepG2 cells to 0–20 μ M 4-HNE for 2 hr, a time frame consistent with the ability of 4-HNE to elicit sublethal cellular oxidative stress (Cheng *et al.*, 2001, Raza and John, 2006). 4-HNE is fully metabolized or has reacted with cellular constituents by 2 hr of exposure (Hartley and Petersen, 1997) and thus later time points were not analyzed. The cells were trypsinized and analyzed for cellular GSH concentrations. The extent of lipid peroxidation was determined by measuring thiobarbituric acid-reactive substances (TBARS) in $10,000 \times g$ cell supernatants prepared in 0.1 mM KCl containing 0.5% butylated hydroxytoluene (BHT) as a reductant to reduce the generation of aldehydes during tissue processing (Buege and Aust, 1978).

2.5 Statistical Analysis

Values reported reflect either the mean (S.E.M.) or mean (S.D.) of triplicate incubations (for biochemical characterization of transfected cell lines) or three replicate cell culture experiments. The effect of 4-HNE on the rates of cell growth in control and hGSTA4 cells was analyzed using two-way analysis of variance (ANOVA) and Bonferoni's post-hoc test. 4-HNE-treatment effects on cellular GSH concentrations and lipid peroxidation in control and hGSTA4 cells were analyzed using one-way ANOVA and Dunnett's *t*-test. All treatment-related effects were considered to be statistically significant at $p \leq 0.05$. Data was analyzed using SigmaStat 2.0 software (Jandel Scientific, San Rafael, CA) or GraphPad Prism version 4 (Hearne Scientific Software, Chicago, IL) for personal computer.

3. Results

A number of hGSTA4 transfected cell lines were selected in the presence of geneticin and analyzed for expression levels of *hGSTA4* mRNA and GST-4HNE activity. After an initial screening, we identified three clonal lines that over-expressed *hGSTA4* mRNA relative to wild type or control cells. These lines were identified as lines A1A, A1D, and A2E. Of the three *hGSTA4* transfected cell lines, A2D exhibited particularly strong expression of *hGSTA4* mRNA relative to the other cell lines and had the highest GST-4-HNE activities (Figure 1). Specifically, the GST-4HNE conjugation activities in the control (insert-free vector transfected), A1A, A1E and A2D cell lines were 29(7), 76(18), 152(15) and 444(32) nmol 4-HNE conjugated/min/mg protein, respectively (Figure 1B). Cell line A2D was selected for further study and are subsequently referred to as "hGSTA4 cells." The *hGSTA4* mRNA levels from non-transfected (wild type) and insert free vector-transfected (control) HepG2 cells did not differ (Figure 1). Similarly, the GSH concentrations in non-transfected cells did not differ from the insert free vector-transfected (control) cells were similar, suggesting that the transfection process did not directly alter the GSH pathway (data not shown). In addition to the high GST-4-HNE conjugating capacity, the hGSTA4 cells also exhibited significantly

higher GST catalytic activities toward CDNB (1.6-fold above controls, table 1), but did not statistically differ from the control cells with regard to GSH peroxidase activities toward CumOOH or H₂O₂ (table 1). The relatively high GST catalytic activities in the hGSTA4 cells were associated with 38% lower basal GSH concentrations than observed in the control cells (table 1).

As observed in figure 2, the hGSTA4 cells exhibited a significant growth advantage relative to control cells in the absence of 4-HNE. The growth advantage in hGSTA4 cells was statistically significant at 8 hr (43% increase relative to control cells) and at 24 hr time points (79% increase relative to control cells, Figure 2). A trend toward increased cell proliferation was observed in hGSTA4 cells exposed to 5–10 μ M 4HNE relative to control cells at similar time points, however, these data were not statistically significant (Figure 2). When challenged with higher concentrations of 4-HNE (15–20 μ M), there was no observable growth advantage of the hGSTA4-transfected cells relative to control cells at any of the time points measured.

To evaluate the functional consequences of hGSTA4 transfection on GSH levels and susceptibility to oxidative damage, hGSTA4 and control cells were exposed to levels of 4-HNE associated with cellular oxidative stress for a period of 2 hr. As observed in Figure 3, exposure to a relatively low dose of 5 μ M 4-HNE for two hours caused a significant decrease in GSH concentrations in control cells, whereas higher doses of 4-HNE stimulated an increase in cellular GSH levels. In contrast, exposure of hGSTA4 cells to 5–15 μ M 4-HNE stimulated an increase in GSH concentrations, with the greatest increase in GSH levels occurring in cells exposed to 10 μ M 4-HNE (3-fold higher GSH levels than controls, Figure 3). Under conditions of high 4-HNE exposure (20 μ M), the GSH levels in hGSTA4 cells did not differ from unexposed hGSTA4 cells (Figure 3).

As observed in Figure 4, the hGSTA4 cells were protected against lipid peroxidation in the presence of all concentrations of 4-HNE. In contrast, exposure to 4-HNE increased the extent of lipid peroxidation in control cells at all doses. Specifically, exposure to 5 μ M, 10 μ M, 15 μ M and 20 μ M 4-HNE resulted in 1.60-, 1.63-, 1.65- and 1.98-fold increases, respectively, in lipid peroxidation when compared to control cells receiving vehicle (Figure 4).

4. Discussion

As discussed, 4-HNE is a cytotoxic endogenous product produced in relatively high amounts during the peroxidation of cell lipids, a common occurrence on exposure to pro-oxidant drugs and xenobiotics. 4-HNE accumulation during the initial stages of oxidative injury can exacerbate the cellular oxidative damage cascade by eliciting further protein and oxidative injury via altering mitochondrial function and redox status which decreases reactive oxygen species clearance (Raza and John, 2005), and also by electrophilic attack on nucleophilic sites of proteins (Esterbauer, 1991). Accordingly, the biochemical pathways that maintain cellular 4-HNE levels are of importance in maintaining cellular function under oxidative stress. The fact that hGSTA4-4 is a polymorphic enzyme (Coppede *et al.*, 2005) that is present at low levels in human tissues raises the intriguing hypothesis that elevation of hGSTA4-4 expression may potentially protect against 4-HNE cellular injury. Others have reported that over-expression of mGSTA4-4, an alpha class GST in mice that exhibits high 4-HNE conjugating activity, protects against cellular oxidative damage *in vitro* (Zimniak *et al.*, 1997). However, human hGSTA4-4 shares only 59% sequence with the mouse A4-4 form at the protein level (Bruns *et al.*, 1999). In addition, mGSTA4-4 and the rat orthologue (rGSTA4-4) are expressed at relatively high levels in tissues relative to the human GSTA4-4 form (Gallagher and Gardner, 2002; Morel *et al.*, 2002). Thus, it was important to target the hGSTA4-4 subclass with regard to potential chemotherapeutic implications for humans.

In the present study, we observed that *hGSTA4*-transfected cells expressed high levels of *hGSTA4* mRNA and an increased capacity to conjugate 4-HNE relative to control cells. While we did not quantitate the levels of *hGSTA4*-4 protein in the transfected cells, the high *hGSTA4* mRNA levels and GST-4-HNE activities in the transfected cells relative to controls indicates translation of the mRNA into functional protein. The fact that the transfected cells had significantly lower basal GSH concentrations than the control cells implies an increased utilization of GSH due to high expression and activity of the *hGSTA4*-4 enzyme and associated demand for GSH. The somewhat higher GSH peroxidase activity toward CumOOH in the *hGSTA4* cells may also have reflected the higher expression of the *hGSTA4*-4 protein, which has catalytic activity towards this substrate (Hubatsch *et al.*, 1998) and which likely placed further demands on constitutive cellular GSH levels. In contrast, GSTs do not catalyze the GSH-mediated reduction of H₂O₂, which is primarily catalyzed by cytosolic selenium-dependent GSH peroxidase (Hayes and Pulford, 1996), and whose activities did not differ among *hGSTA4* and control cells.

Our human HepG2 cells over-expressing *hGSTA4*-4 exhibited a growth advantage relative to control cells by analysis of cell proliferation using the Alamar Blue assay. The growth advantage was marked in the absence of 4-HNE, but declined under increasing concentrations of 4-HNE. These data suggest that other factors such as removal of GSH-4-HNE conjugates by GSH conjugate efflux transporter proteins may play an important role in cell protection against 4HNE injury (Morrow *et al.*, 1998, 2000). It also must be pointed out that although the Alamar Blue assay is widely employed in toxicological studies to assess cell viability and cell proliferation rates, the assay provides a relative measure of cell proliferation, but not an absolute one. In particular, others have noted that the fluorescent product of Alamar Blue in cell media can sometimes lead to an overestimation of cell population (O' Brien *et al.*, 2000).

Interestingly, exposure to 4-HNE elicited an increase in GSH concentrations in both the control and the *hGSTA4* cells, although the dose-response of GSH induction in the two cell lines was very different. Others have shown that 4-HNE exposure can increase cellular GSH through induction of glutamylcysteine ligase (GCL) activity by transcriptional activation of the *GCL* catalytic (*GCLc*) and modulator (*GCLm*) subunit mRNAs (Dickinson *et al.*, 2002). The fact that cellular GSH concentrations in *hGSTA4* cells exposed to 15 μ M 4-HNE did not differ from vehicle-exposed *hGSTA4* cells, and were similar to unexposed *hGSTA4* cells when exposed to 20 μ M 4-HNE, indicates that the increased 4-HNE-GSH conjugation in the presence of high concentrations of 4-HNE may have surpassed the ability of the *hGSTA4* cells to synthesize GSH. The observed loss of GSH at a low level 4-HNE exposure (5 μ M) in the control cells is consistent with a report by Malone and Hernandez (2006) who observed a significant depletion of GSH in astrocytes exposed to 4-HNE for 1–3 hrs. Collectively, our data suggests that GST expression may be an important co-regulator of GSH levels in cells exposed to 4-HNE.

Although we did not measure 4-HNE concentrations in unexposed or wild type HepG2 cells, others have reported cellular basal 4-HNE levels of approximately 1–5 μ M (Esterbauer *et al.*, 1991). When challenged with exogenous 4-HNE, the cellular antioxidant defenses in the control HepG2 cells could not protect against lipid peroxidation. In contrast, even at the highest dose of 4-HNE tested in our study (20 μ M), the *hGSTA4* cells were still protected against lipid peroxidation, despite exhibiting relatively lower levels of GSH compared to control cells. It is highly possible that under conditions of chronic or higher 4-HNE exposure that the GSH biosynthetic capacity in the *hGSTA4* transfected cells may not be able to sustain protection against 4-HNE-mediated lipid peroxidation. We did not expose cells to 4-HNE at concentrations exceeding 20 μ M due to a lack of physiological relevance, as higher levels of 4-HNE are typically generated only under conditions of severe oxidative stress (Esterbauer *et al.*, 1991).

It must be pointed out that the GST-4-HNE activity levels in our hGSTA4 transfected cells was relatively high, and as discussed, hGSTA4-4 is not the only biochemical pathway for protection against 4-HNE-oxidative injury in human cells. Accordingly, of interest would be assessing the contribution of other 4-HNE metabolizing enzymes in protecting against 4-HNE oxidative damage our cell system. In particular, the oxidative production of 4-hydroxy-2-nonenic acid (HNA, an ALDH metabolic product) along with HNE-GSH adducts in rat Kupffer cells indicates a combined response of oxidative metabolism via the ALDH enzyme system and GST conjugation in mediating 4-HNE oxidative injury in liver cells (Luckey and Petersen, 2001). Other factors such as removal of GSH-4-HNE conjugates by GSH conjugate efflux transporter proteins can play an important role in cell protection against 4-HNE injury (Morrow *et al.*, 2000). In this regard, both the multidrug resistance-associated proteins MRP1 and MRP2 (Morrow *et al.*, 2000; Ji *et al.*, 2004) and Ral-binding GTPase-activating protein RLIP76 (Sharma *et al.*, 2001) are important cellular transporters for the removal of GSH conjugates. Of these transporters, RLIP76 may be the predominant pathway for removing GSH-HNE and other GSH-electrophile conjugates (Sharma *et al.*, 2001). It would thus be of interest to observe if co-transfection of cells with both hGSTA4 and RLIP76 further enhances cellular resistance against 4-HNE-mediated oxidative injury, especially at high 4-HNE concentrations (>20 μ M).

In conclusion, we have shown that over-expression of hGSTA4 confers high 4-HNE conjugation activity and protects against 4-HNE mediated lipid peroxidation. It should be noted that our laboratory, as well as others, typically use a racemic 4-HNE mixture of (*R*)- and (*S*)-enantiomers in cell studies, as this mixture is formed in biological systems (Gueraud *et al.*, 2005). However, the (*R*)- and (*S*)-enantiomers can differ with respect to their biological properties and there may be differences in the reduction and retro-Michael conversion steps of the metabolism between the conjugates originating from the two enantiomers (Gueraud *et al.*, 2005). Additional studies regarding the specific activities of hGSTA4-4 and other human GST isoforms towards these two enantiomers, and the contributions of competing aldehyde metabolizing enzymes and GST-electrophile transporters will help shed further light on the chemoprotective utility of GST over-expression in human cells.

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Figure 1.

The effect of transfecting HepG2 cells with hGSTA4 on (A) *hGSTA4* mRNA expression by semi-quantitative RT-PCR. β -globin mRNA is presented as a housekeeping gene in the reactions. (B) GST-4-HNE conjugating activity in cell lysates. In panel B, asterisks indicate significant differences in GST-4-HNE activities from no- insert controls (control cells) at $p \leq 0.05$. Baseline GST-4-HNE activities in wild-type HepG2 cells (WT) are included for comparative purposes.

Figure 2.

Effect of 4-HNE on cell proliferation in control and hGSTA4-transfected cells. Cells were incubated in 96 well plates in the presence of 5–20 μM 4-HNE (or 0.05% ethanol control, 0 μM) and 10% Alamar blue. Alamar blue fluorescence was measured at 1, 2, 4, 8, and 24 hr after initial incubation as described in methods. Values represent the % increase in Alamar blue fluorescence from initial 1 hr post-exposure measurements, and data reflect the mean (SEM) of three experiments. Asterisks indicate significantly greater rate of cell proliferation rates in hGSTA4 cells relative to control (no-insert vector transfected) cells at $p \leq 0.05$ using two-way ANOVA and Bonferroni's post-hoc comparisons test.

Figure 3.

Effect of 4-HNE on GSH concentrations in control and hGSTA4-transfected cells. HepG2 cells were incubated in the presence of 5–20 μ M 4-HNE (or 0.05% ethanol control) and assayed at 2 hr for GSH concentrations. Data represent mean $\pm$ standard deviation of three replicate experiments and asterisks indicate significant treatment differences in GSH concentrations from 0 μ M control for each cell type at $p \leq 0.05$ using one-way ANOVA and the Dunnett's t -test.

Figure 4.

Effect of 4-HNE on the extent of lipid peroxidation in control (triangles) and hGSTA4-transfected cells (squares). Cells were incubated in the presence of 0–20 μM 4-HNE (or 0.05% ethanol control) and assayed at 2 hr for the extent of lipid peroxidation. Data represents mean $\pm$ SEM of three replicate experiments and asterisks indicate significant differences in lipid peroxidation among control and hGSTA4 cells using one-way ANOVA at $p \leq 0.05$.

Comparative GSH-dependent catalytic activities and GSH concentrations in control (no-insert vector transfected) and hGSTA4-transfected cells* .

Cell line	GSH nmol/g	GST-4HNE nmol/min/mg	GST-CDNB nmol/min/mg	GSH-CumOOH nmol/min/mg	GSH-H ₂ O ₂ nmol/min/mg
control	1300(92)*	29(7)	17(1)	39(5)	14(1)
hGSTA4	801(64)	444(31)*	28(1)*	53(8)	13(1)

* data represents the mean (SEM) of three cellular preparations with significant differences noted at $p<0.05$.