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Differential protection by human glutathione S-transferase P1 against cytotoxicity of benzo[a]pyrene, dibenzo[a,l]pyrene, or their dihydrodiol metabolites, in bi-transgenic cell lines that co-express rat versus human cytochrome P4501A1

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are activated by cytochrome P450 (CYP) isozymes, and a subset of the reactive metabolites generated is detoxified via conjugation with glutathione (GSH) by specific glutathione S-transferases (GSTs). We have used V79MZ cells stably transfected with either human or rat cytochrome P4501A1 (CYP1A1), alone or in combination with human GSTP1 (hGSTP1), to examine the dynamics of activation versus detoxification of benzo[a]pyrene (B[a]P), dibenzo[a,l]pyrene (DB[a,l]P), and their dihydrodiol metabolites. The cytotoxicity of B[a]P or DB[a,l]P was 9- to 11-fold greater in cells expressing human, as compared to rat CYP1A1, despite similar enzymatic activities. Co-expression of the hGSTP1 with the hCYP1A1 conferred 16-fold resistance to B[a]P cytotoxicity, compared to only 2.5-fold resistance when hGSTP1 was co-expressed with rat CYP1A1. The lower B[a]P cytotoxicity in the cells expressing rat CYP1A1, and weaker protection by hGSTP1 co-expression in these cells, were attributable to the much lower fraction of B[a]P metabolism via formation of the 7,8-dihydrodiol intermediate by the rat CYP1A1 compared to hCYP1A1. Resistance to the DB[a,l]P cytotoxicity conferred by hGSTP1 expression was also greater in cells co-expressing hCYP1A1 (7-fold) as compared to cells co-expressing rCYP1A1 (< 2-fold). Resistance to B[a]P conferred by hGSTP1 was closely correlated with the activity level in two clonal transfectant lines with a 3-fold difference in hGSTP1-1 specific activity. Depletion of GSH to 20% of control levels via pretreatment with the *de novo* GSH biosynthesis inhibitor buthionine sulfoximine reduced the protection against B[a]P cytotoxicity by hGSTP1 from 16-fold to 5-fold, indicating that catalysis of conjugation with GSH, rather than binding or other effects, is responsible for the resistance. The cytotoxicity of the dihydrodiol intermediates of B[a]P or DB[a,l]P was much greater, and similar in cell lines expressing either human or rat CYP1A1. Again, however, the protection conferred by hGSTP1 co-expression was 2- to 5-fold greater in cells with hCYP1A1 than with rCYP1A1 expression. These results indicate that GST expression can effectively limit cytotoxicity

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following activation of B[a]P by human or rat CYP1A1, but is less effective as a defense against exposure of cells to the intermediate metabolite B[a]P-7,8-dihydrodiol.

Keywords

glutathione S-transferase; cytochrome P-450; benzo[a]pyrene; dibenzo[a,l]pyrene; polycyclic aromatic hydrocarbon; carcinogen detoxification; transfection

INTRODUCTION

Detoxification of hydrophobic xenobiotics often involves metabolic reactions and transport processes that modify their solubility and chemical reactivity, and alter their biological fate. The initial intracellular oxidative metabolism of endogenous or exogenous procarcinogens by cytochrome P-450 (CYP) and other oxygenases can generate highly reactive chemical species that may be cytotoxic or genotoxic [1]. Indeed, many carcinogens such as the polycyclic aromatic hydrocarbons (PAH) benzo[a]pyrene (B[a]P) or dibenzo[a,l]pyrene (DB[a,l]P) have little toxicity until they are activated via “phase I” pathways that produce reactive metabolites. This initial biotransformation is often necessary to generate precursors of sufficient reactivity to serve as substrates for “phase II” reactions that render the conjugated product more water-soluble, less reactive and hence, less toxic or mutagenic [2]. In addition to quenching the reactivity of the products of phase I reactions, conjugation often results in a product that is flagged for export by a “phase III” cellular active transport process that recognizes the conjugate as a species targeted for removal from the cell [3]. Morrow et al. were the first to demonstrate that removal by MRP-1 of the glutathione conjugate of a carcinogen, 4-nitroquinoline-1-oxide (NQO) was necessary to enable protection by transfected human glutathione S-transferase P1 (hGSTP1) against the cytotoxicity and DNA adduct formation by NQO [4]. Thus, the dynamic competition between the activation reactions and the subsequent detoxification steps, the reactivity and half-life of the intermediary metabolites, and cellular disposition of the reaction products are all important factors that may determine the consequences of exposure to PAHs. The recent findings that CYP1A1 knockout mice are more, rather than less sensitive to oral B[a]P lethality are intriguing in this regard, and reinforce the importance of pharmacokinetics in determination of toxicity. The conclusion of these studies was that systemic loss of CYP1A1 expression resulted in a reduced B[a]P clearance rate and consequently greater damage to the sensitive hematopoietic system due to the longer exposure to high circulating B[a]P levels [5].

Polycyclic aromatic hydrocarbons (PAHs) are activated by cytochrome P450 (CYP) isozymes, and a subset of the reactive metabolites generated is detoxified via conjugation with glutathione (GSH) by specific glutathione S-transferases (GSTs). Several different activation pathways of PAHs are known, and the CYP isozymes most involved in the activation of PAHs have been identified as CYP1A1, CYP1A2, CYP1B1 and CYP3A4 [6–8]. The most cytotoxic and mutagenic metabolites are the bay and fjord region diol-epoxides, generated primarily via the carcinogen-inducible isozymes CYP1A1 [9,10] and CYP1B1 [11,12]. These CYPs metabolize PAH carcinogens to bay and fjord region diol-epoxides in a stereoselective manner, typically resulting in two pairs of diastereomeric (+/–) *syn* and (+/–) *anti* diol-epoxides [13,14].

Glutathione S-transferases (GSTs) are thought to play an important role in cellular defenses against the toxic or mutagenic effects of reactive electrophiles by catalyzing conjugation of electrophilic centers of reactive species with the nucleophilic thiol group of glutathione (GSH) [15]. The GST-catalyzed conjugation with GSH is proposed to provide a major component of cellular defense against PAH diol-epoxides [16]. Different GSTs display variable catalytic efficiencies towards activated PAH metabolites [17]. Previous studies from this lab indicated

that protection by GSTs against intrinsically reactive electrophiles can be determined by either the substrate specificity or the level of the expressed GST isoform [18,19]. However, little is known regarding the relative dynamics of competing activation versus detoxification pathways expressed in the same cell, for determining the toxicity of compounds that require an activation pathway. Furthermore, the conventional roles in activation versus detoxification of the respective phase I CYP and phase II GST pathways have been challenged by studies in knockout mouse models that revealed paradoxical resistance to B[a]P toxicity in mCYP1A1 null mice [20], and paradoxical resistance to acetaminophen toxicity in mGSTP1 null mice [21]. Here we report studies with V79 cells stably transfected with either human or rat CYP1A1, in comparison with derivative lines stably super-transfected with hGSTP1, extending preliminary results for the parent PAHs included previously in a report of a symposium presentation [22]. These cell lines were used to examine the relative importance of the activation by rat versus human CYP1A1 isozymes, in competition with opposing detoxification by hGSTP1 for reduction of the cytotoxicity induced by exposure to B[a]P, DB[a,l]P or their dihydrodiol intermediate metabolites.

MATERIALS AND METHODS

Materials and Chemicals

Caution: The PAHs described in this publication are chemical carcinogens and must be handled with care as outlined in the National Cancer Institute guidelines

—Cell culture medium was purchased from Gibco/BRL (Grand Island, NY). Fetal bovine serum was purchased from Cellgro/Mediatech (Herndon, VA). B[a]P was purchased from Sigma (St. Louis, MO). DB[a,l]P, DB[a,l]P-11,12-dihydrodiol, and the pure enantiomers (+)- and (–)-B[a]P-*trans*-7,8-dihydrodiol were purchased from Midwest Research Institute (Lexena, KS). All other chemicals were analytical grade from Fisher Scientific (Atlanta, GA) or Sigma (St. Louis, MO).

Cell culture and Cell lines

Parental V79MZ cells, and V79MZh1A1 cells, expressing transfected human CYP1A1 [14] or V79MZr1A1 cells, expressing transfected rat CYP1A1 (originally designated as XEM2) [23] were grown and maintained in DMEM medium supplemented with 5% fetal bovine serum and selected with 400 µg/ml G418 at 37°C in a 5% CO₂ atmosphere. Cells were passaged at 1:20 dilution every 2–3 days. The human glutathione-S-transferase P1 cDNA was inserted downstream of a cytomegalovirus promoter in the pCEP4 expression vector as previously described [24]. The V79MZr1A1 and V79MZh1A1 cell lines, expressing rat or human CYP1A1, respectively, were stably transfected with this vector by the calcium phosphate co-precipitation method, and clonal colonies picked after 7–10 days of selection in 0.4 mg/ml hygromycin as previously described [25].

Ethoxyresorufin O-deethylase (EROD) assay

Cells were plated on 100 mm plates and harvested by scraping into cold PBS + 5 mM EDTA, pelleted, and the cell pellets sonicated in 50 mM Tris, pH 7.4 and centrifuged at 1000 × g to remove cell debris. CYP1A1 activities were measured in the crude supernatant by the method of Burke et al. [26]. Total cellular protein (150–300 µg) was added to a total volume of 1 ml containing 50 mM Tris-HCl, 25 mM MgCl₂, 5 mM glucose-6-phosphate, 50 U/ml Glucose-6-phosphate dehydrogenase, 10 µM dicoumarol, 100 µM NADPH, and initiated by the addition of 5 µM ethoxyresorufin. Samples were incubated at 37°C for 1 hour and the reaction was stopped by the addition of 1 ml ice cold methanol. Fluorescence was measured on a Perkin-Elmer LS-3B spectrofluorometer with excitation at 522 nm and emission at 586 nm, and resorufin determined by comparison to a standard curve established with solutions of known resorufin concentration.

B[a]P Metabolite analysis

Transgenic cell lines (V79MZh1A1 or V79MZr1A1) were plated, exposed to B[a]P, and the medium harvested and analyzed by reverse-phase HPLC as previously described [27]. Results are expressed as a percent of the total metabolites detected with each cell line.

GST assay

The method used is a modification of the method of Habig [28]. Briefly, 2–20 μ l of sample was assayed at 23°C in a solution of 0.1M potassium phosphate, pH 6.5 and 1 mM GSH. The reaction was initiated with 1 mM (final) 1-chloro 2,4 dinitrobenzene (CDNB), and the change in absorbance was monitored at 340 nm for 90 sec. Activity was calculated from the $\Delta A/\text{min}$ and extinction coefficient, reported in nmol/min/mg protein. Protein concentrations were determined by the bicinchoninic acid protein assay (Pierce, Rockford, IL) with bovine serum albumin as standard.

Cytotoxicity assays

Cells were plated in the absence of G418 selection at a density of 250 cells/well on 96-well plates and allowed to attach for 24 hrs. Various concentrations of PAH were added (in ethanol, 0.1% final solvent concentration) at concentrations selected to span at least 3-fold above and below the IC_{50} value. Glutathione depletion studies included either 5 μ M or 10 μ M buthionine sulfoximine (BSO), an irreversible inhibitor of GSH synthesis, added 8 h after plating and 16 h prior to B[a]P addition, which was previously determined to deplete GSH levels > 50% or > 80%, respectively, without significant cytotoxicity (data not shown). Cells were exposed continuously for 4 more days (grown to approximately 90% confluence in vehicle control wells), then rinsed with PBS and fixed by addition of cold 5% trichloroacetic acid. Plates were stained with 0.4% sulforhodamine B (SRB) in 1% acetic acid for 10 minutes, rinsed five times, dried, and the SRB was resolubilized in 0.1 M Tris base (pH 10). Plates were read on a microplate spectrophotometer (Molecular Devices, Sunnyvale, CA) at 560 nm, as an indirect measure of cell number, compared as a percentage of staining in the untreated control plate.

RESULTS

Transgenic cell lines

Activity of hGSTP1-1 in V79MZh1A1 was 3181 ± 429 (clone Pi-23) and 992 ± 131 (clone Pi-25) nmol/min/mg (Table 1). These activities were comparable to the V79MZr1A1 cell line transfected with hGSTP1-1 (2538 ± 164 nmol/min/mg). The background GST activities were 209 nmol/min/mg or less in the V79MZ, V79MZr1A1, and V79MZ h1A1 cell lines, due to basal expression of hamster GSTP1. This pi class hamster GST isozyme has been shown to have negligible activity for conjugation of B[a]P-7,8-diol-9,10-epoxide (BPDE) in V79 cells [29]. The CYP1A1 activities measured in the EROD assay were comparable in the cell lines within each group, and differed by less than 2-fold between the rat and human CYP1A1-expressing groups (Table 1). This is consistent with the similar activities originally reported for the V79MZr1A1 and V79MZh1A1 cell lines using both the EROD and aryl hydrocarbon hydroxylase (AHH, or B[a]P hydroxylase) assays [14]. The EROD activities of both rat and human CYP1A1 in these cell lines were previously determined to be comparable to the activity measured in non-induced rat liver [14,23]. The parental V79MZ cell line has only low background activity in the EROD assay, and these cells have undetectable activity in the AHH assay and are insensitive to the mutagenic or cytotoxic effects of B[a]P even at high concentrations and prolonged exposures [23,30–32].

Activation of B[a]P or DB[a,l]P and reversal of toxicity by hGSTP1

Rat CYP1A1 expression caused a 3-fold enhancement of B[a]P cytotoxicity relative to the non-expressing control V79MZ cells. This toxicity was almost completely reversed by co-expression of hGSTP1, which conferred a 2.5-fold resistance to B[a]P cytotoxicity (Table II). Cells expressing human CYP1A1 activated B[a]P much more effectively, exhibiting sensitivity to toxicity 27-fold greater than the control V79MZ cells ($IC_{50} = 0.27 \mu M$ versus $7.5 \mu M$, respectively), and 9-fold greater sensitivity to B[a]P toxicity than the V79MZr1A1 cells expressing rat CYP1A1 ($IC_{50} = 2.4 \mu M$). Cells expressing both human CYP1A1 and human GSTP1 (V79MZh1A1/hGSTP1-23) were largely protected against toxicity of B[a]P, with ~16-fold resistance relative to cells expressing only hCYP1A1. The 16-fold protection with hGSTP1 reversed the toxicity due to activation by hCYP1A1 to within 2-fold difference from the control V79MZ cells ($IC_{50} = 4.2 \mu M$; Table II). An intermediate level of hGSTP1 activity in the hCYP1A1-expressing cells (V79MZh1A1/hGSTP1-25) conferred a proportional (6-fold) degree of protection against B[a]P (Table II and Fig. 1). There was a high degree of correlation between the specific activity of GST expressed and the protection against B[a]P cytotoxicity, as shown by the linear proportionality with the IC_{50} values in Figure 1 (least squares correlation coefficient $R^2 = 0.99$). Thus, under these conditions, the expression level of GSTs governed the cytotoxicity of B[a]P activated via transfected hCYP1A1 in a manner commensurate with the GST expression level, and this resulted in blocking most of the lethality at the highest level of hGSTP1 expression.

The potent carcinogen DB[a,l]P was almost two orders of magnitude more cytotoxic than B[a]P in both V79MZr1A1 and V79MZh1A1 cell lines, and was activated to cytotoxic species at least an order of magnitude more effectively by hCYP1A1 ($IC_{50} = 2.9 \mu M$) than by rCYP1A1 ($IC_{50} = 33 \mu M$) (Table III). Co-expression of hGSTP1 conferred strong (7-fold) resistance to DB[a,l]P in cells expressing hCYP1A1, but much weaker resistance (< 2-fold) with rCYP1A1 expression (Table III).

GST protection against B[a]P or DB[a,l]P dihydrodiol intermediates

Cells were also tested for sensitivity to the intermediate metabolites (+)- or (-)-B[a]P-7,8-dihydrodiol, and DB[a,l]P-11,12-dihydrodiol. Expression of the rat or human CYP1A1 isozymes conferred a similar degree of activation for each of the dihydrodiol metabolites, with over two orders of magnitude enhancement of toxicity relative to the V79MZ parental control cells. However, there were differences in the protection by hGSTP1 depending on the species of origin of the CYP1A1 expressed, again with greater protection in the cells expressing hCYP1A1. In the rat CYP1A1 expressing cells, hGSTP1 provided 2-fold or less protection against any of the intermediate B[a]P-7,8-diols or DB[a,l]P-11,12-dihydrodiol (Table IV and Table V). Protection against cytotoxicity of the (+)-B[a]P-7,8-dihydrodiol in the human CYP1A1-expressing cells was 5-fold, compared to 2.4-fold protection against the (-)-B[a]P-7,8-dihydrodiol (Table IV). Thus the products of human CYP1A1 activation of (+)-B[a]P-7,8-dihydrodiol, i.e. (+)-*syn*- and/or (-)-*anti*-B[a]P-7,8-diol-9,10-epoxide (BPDE) enantiomers, are detoxified twice as effectively by hGSTP1 as the (-)-*syn*- and/or (+)-*anti*-BPDE enantiomers formed from (-)-B[a]P-7,8-dihydrodiol. In contrast to the 1.9-fold protection by hGSTP1 in cells expressing rat CYP1A1, the cytotoxicity of DB[a,l]P-11,12-dihydrodiol was reduced by 9.2-fold by hGSTP1 when co-expressed with human CYP1A1 (Table V).

Differences in rat versus human CYP1A1 B[a]P metabolite profiles

The greater protection by hGSTP1 against B[a]P, DB[a,l]P or the intermediate dihydrodiols in cells expressing human as compared to rat CYP1A1 are presumably due to differences in the relative rates of activation of these PAHs to downstream metabolites. This possibility was suggested by HPLC analysis in cell culture medium of soluble products of the CYP1A1-

catalyzed activation at several of the known major positions of initial oxidation on the B[a]P polycyclic aromatic ring structure [27]. Most notably, the total B[a]P-7,8-oxidation fraction – representing the sum of the B[a]P-7,8-dihydrodiols formed from B[a]P-7,8-oxide plus the 7,8,9,10-tetrols, formed from hydrolysis of B[a]P-7,8-diol-9,10-epoxide (BPDE) – were 4- to 5-fold higher in the medium of the V79MZh1A1 cells than the V79MZr1A1 cells (Fig. 2). The 9,10-oxidation products, which also contribute to the 7,8,9,10-tetrol pool, were 2-fold higher with V79MZh1A1 cells than with V79MZr1A1 cells. Further, the rCYP1A1-expressing cells produced a higher fraction of the less toxic 4,5-oxidation products, and the relatively much less toxic 3-hydroxylation products, neither of which are likely to be detoxified by hGSTP1.

Dependence on GST catalysis

The ability of GSTs to bind hydrophobic molecules was the first described function for this family of gene products, and hence it was initially termed “ligandin”, before the catalytic activity was discovered [33]. Since this binding activity could also serve a defensive role against activated PAH metabolites independent of the conjugation function, we tested the protection against B[a]P cytotoxicity by hGSTP1 after depletion of GSH, induced by pretreatment of cells with buthionine sulfoximine (BSO), a selective inhibitor of -glutamylcysteine ligase, the rate-limiting enzyme in *de novo* GSH biosynthesis [34]. Pretreatment (16 hr) and continued coincubation with 5 μ M BSO, which resulted in approximately 50% depletion of the co-substrate GSH (not shown), had little effect on the fold-resistance conferred by hGSTP1 against B[a]P cytotoxicity in the hCYP1A1-expressing cell lines. However, at 10 μ M BSO, a concentration that resulted in greater than 80% depletion of cellular GSH (not shown), the protection by GSTP1 expression against B[a]P cytotoxicity was reduced from 16-fold to 5-fold (Figure 3). Thus, reducing intracellular GSH to a concentration that was likely below the K_m of hGSTP1-1 for GSH resulted in loss of over half of the protective capacity of hGSTP1-1 for detoxification of B[a]P metabolites produced by hCYP1A1. This supports the conclusion that GSTP1-1 catalysis of conjugation of B[a]P metabolites was necessary for the protective effect, and also demonstrates that even low residual levels of GSH still supported significant detoxification of activated B[a]P metabolites by hGSTP1-1.

DISCUSSION

Human CYP1A1 catalyzes efficient activation of B[a]P to more reactive metabolites, as evident by the strong (27-fold) enhancement of cytotoxicity in V79MZ cells constitutively expressing transfected hCYP1A1 compared to non-expressing V79MZ control cells. We have previously shown in a preliminary report that hGSTP1 provided better protection against B[a]P or DB[a,l]P when activated by human as compared to rat CYP1A1 [22]. The present studies now also indicate that hGSTP1 also protects better against the dihydrodiol metabolites of B[a]P or DB[a,l]P when activated by hCYP1A1 than when activated by rCYP1A1. This is likely due to the different B[a]P metabolite profile generated by hCYP1A1, which results in a greater proportion of ultimate conversion via the 7,8-oxidation pathway to BPDE than with the rat CYP1A1. Activation by the rat CYP1A1 thus was much less effective for generation of cytotoxic metabolites, as indicated by the 14-fold weaker cytotoxicity of B[a]P in the cells expressing transfected rat as compared to human CYP1A1. The weaker B[a]P toxicity is likely due to initial oxidation by rCYP1A1 of B[a]P preferentially to much less toxic intermediates such as 3-hydroxy-B[a]P and B[a]P-4,5-dihydrodiol, and production of lower amounts of B[a]P-7,8-dihydrodiol [27], as illustrated in Figure 2. The greater conversion to the more toxic and mutagenic BPDE thus enables a greater fold-protection since it is also a very good substrate for conjugation by hGSTP1 [17]. Further supporting this explanation, the B[a]P-7,8-dihydrodiols have similar cytotoxic potency in cells expressing either human or rat CYP1A1, consistent with the interpretation that the differential sensitivity to B[a]P metabolism likely results primarily from a greater rate of formation by hCYP1A1 than rCYP1A1 of the initial

epoxide(s) and hydrolysis to the corresponding dihydrodiols. The CYP-catalyzed oxidation of B[a]P in the “bay region” yields the most toxic and mutagenic metabolites, whereas some other PAHs such as DB[a,l]P generate their most electrophilic products via modifications at a sterically hindered “fjord region”. Metabolism of DB[a,l]P by hCYP1A1 resulted in more than 300-fold enhancement of cytotoxicity, compared to 30-fold activation by rCYP1A1.

The cells expressing rat CYP1A1 together with hGSTP1 exhibited approximately 2.5-fold protection by the GST against B[a]P compared to the cells expressing rat CYP1A1 only; nevertheless, this constituted reversal of virtually all of the enhancement of B[a]P toxicity engendered as a result of rat CYP1A1 expression. Similarly, GST-catalyzed conjugation provided a strong cellular defense (16-fold protection) against the much greater cytotoxicity of the B[a]P reactive metabolites produced via activation by human CYP1A1, again reversing most of the cytotoxicity. An important inference from the near-complete reversal of the CYP1A1-mediated enhancement of B[a]P toxicity at the highest hGSTP1 expression level, is that co-expression of GST can be the dominant factor controlling the toxicity of B[a]P that is activated by hCYP1A1 or rCYP1A1. In contrast, even with the 7-fold reduction of DB[a,l]P cytotoxicity by hGSTP1, the IC₅₀ remains more than 50-fold lower than in the cells without any CYP expression. Thus, with DB[a,l]P, and with all of the dihydrodiol intermediate metabolites, the phase I activation is the dominant factor controlling the cytotoxicity.

The fold-protection against B[a]P cytotoxicity was closely proportional to the levels of GST activity in the hGSTP1-transfected cell lines, with a correlation coefficient of near unity (Figure 1). The relationship with the level of activity expressed was clearly evident in the 3-fold difference in protection in the two cell lines (V79MZh1A1/hGSTP1-25 and V79MZh1A1/hGSTP1-23), exactly correlating with a 3-fold difference in hGSTP1-1 activity ($R^2 = 0.99$). This correlation implies that the protection against hCYP1A1-generated B[a]P metabolites by hGSTP1-1 is not yet maximal even in the highest GST expressing cell line used in these experiments. This activity (3181 mU/mg) is comparable to GST expression levels observed in placenta and liver, but higher than in the majority of epithelial tissues. Therefore, induction of GST expression by chemopreventive agents is likely to confer a degree of protection against B[a]P that is proportional to the GST activity in most tissues in which B[a]P activation occurs via CYP1A1 or similar oxidation pathways. Preliminary studies in our lab indicated that coexpression of transfected hGSTP1 can also provide protection against the cytotoxicity of B[a]P activated by transfected hCYP1B1 (S. Kabler and S. Ahmad, unpublished observation).

The initial activation of circulating B[a]P to intermediate metabolites has been proposed to occur largely in liver, followed by redistribution of the stable dihydrodiols to other tissues where they may be further activated to the most reactive diol-epoxide species [35]. Hence it is of interest to examine the role of GST in protection against B[a]P-7,8-dihydrodiols, the precursors of the highly cytotoxic and mutagenic benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE) metabolites. Expression of either rat or human CYP1A1 resulted in potent activation of the B[a]P-7,8-dihydrodiol enantiomers, resulting in a comparable enhancement of toxicity by more than two orders of magnitude (Table IV). The similar cytotoxicity of the two B[a]P-7,8-dihydrodiol enantiomers was consistent with observations in the original report describing B[a]P metabolism in this cell line [14]. However, we and others have noted higher mutagenicity when the V79MZh1A1 cell line is exposed to (–)-B[a]P-7,8-dihydrodiol than (+)-B[a]P-7,8-dihydrodiol [14,32,36], as expected due to the reported preferential formation by hCYP1A1 of the more mutagenic (+)-anti-BPDE from (–)-B[a]P-7,8-dihydrodiol [37]. Taken together, this suggests that the target of cytotoxicity is not limited to DNA damage, and has a different metabolite specificity.

The twofold greater protection against cytotoxicity of (+)-B[a]P-7,8-dihydrodiol as compared to (–)-B[a]P-7,8-dihydrodiol by expression of hGSTP1 is consistent with the two-fold higher

catalytic efficiency of the hGSTP1-1 isozyme for conjugation of (+)-*syn*-BPDE as compared to (+)-*anti*-BPDE [17], the predominant hCYP1A1 metabolites of (+)-B[a]P-7,8-dihydrodiol and (–)-B[a]P-7,8-dihydrodiol, respectively [37]. The reversal by GST expression ranged only up to 5-fold with the (+)-B[a]P-7,8-dihydrodiol, and 2.4-fold with the (–)-B[a]P-7,8-dihydrodiol, in contrast to the results with the parent compound B[a]P. The greater enhancement of cytotoxicity due to activation of the proximal intermediate precursor B[a]P-7,8-dihydrodiols by hCYP1A1 was much stronger than the opposing protection by hGSTP1, and hence in this case activation, rather than detoxification, was the more dominant aspect of the metabolic phenotype. This lends further support to the notion that the rate-limiting initial oxidation of B[a]P allows for more effective protection by GST expression by acting to limit the rate of production of the B[a]P-7,8-dihydrodiols and their cytotoxic metabolites. Interestingly, although the rat and human CYP1A1 activated both (+)- and (–)-B[a]P-7,8-dihydrodiols, and also the DB[a,l]P-11,12-dihydrodiols to ultimate metabolites that resulted in similarly potent toxicity, the rCYP1A1 products were in every case less effectively detoxified by hGSTP1 (1.5– to 2–fold) than those produced by the human CYP1A1 (2.4– to 9.2–fold). Although the hGSTP1-1 activity expressed in V79MZh1A1/hGSTP1 cell line was 25% higher, this does not fully account for the more than 2–fold greater protection, and the reason for the remaining difference is presently unknown.

These results illustrate the utility of hGSTP1 expression in cellular defense against cytotoxicity of B[a]P or DB[a,l]P, as well as their principal dihydrodiol metabolites. Protection was more effective against toxicity of the parent compound B[a]P than against the B[a]P-7,8-dihydrodiol metabolites. The reasons for this difference are likely both the rate limitation for activation imposed at the first CYP1A1 epoxidation step and the high reactivity and instability of the ultimate metabolite BPDE formed from B[a]P-7,8-dihydrodiol. This may have important implications for the metabolism of B[a]P at the physiologic level, since the liver has far more CYP activity available for the first epoxidation step than does the lung, a primary target tissue. Thus, the B[a]P-7,8-dihydrodiol, a stable metabolite, can be formed in the liver and recirculated to the lung, where the second epoxidation to the highly toxic and mutagenic diol-epoxide metabolite would occur more readily, since lung constitutively expresses hCYP1B1 which readily catalyzes this second step [11]. These studies also highlight and reinforce the substantial body of information on problems associated with using rodent models that may differ considerably from the corresponding human tissues in their metabolic substrate specificities for prediction of human responses to carcinogens [38].

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Abbreviations

B[a]P	benzo[a]pyrene
BCA	bis-cinchoninic acid
BPDE	benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide
BSO	

	buthionine sulfoximine
CDNB	1-Cl-2,4-dinitrobenzene
DB[al]P	dibenzo[a,l]pyrene
EDTA	ethylene diamine tetraacetate
EROD	ethoxyresorufin O-deethylase
GSH	glutathione
hGSTP1	human pi class glutathione S-transferase-P1 cDNA
hGSTP1-1	homodimeric human pi class GSTP1 isozyme
hCYP1A1	human cytochrome P-4501A1
hGSTP1	human glutathione S-transferase pi
PAH	polycyclic aromatic hydrocarbon
P450	cytochrome P-450
rCYP1A1	rat cytochrome P-4501A1
V79MZh1A1	V79MZ cells expressing human CYP1A1
V79MZh1A1/hGSTP1-23	clone 23 of V79MZh1A1 cells co-expressing stably transfected human GSTP1
V79MZh1A1/hGSTP1-25	clone 25 of V79MZh1A1 cells co-expressing stably transfected human GSTP1
V79MZr1A1	V79MZ cells expressing rat CYP1A1
V79MZr1A1/hGSTP1-33	clone 33 of V79MZr1A1 cells co-expressing human GSTP1

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Figure 1. Correlation between GST expression level and fold-protection against B[a]P cytotoxicity in cell lines expressing stably transfected hCYP1A1, without or with stably transfected hGSTP1 Cytotoxicity and GST specific activity assays were performed as described in Materials and Methods. The IC₅₀ values from Table II are plotted versus GST specific activity.

Abbreviations: V79MZh1A1, V79MZ cell line expressing only stably transfected hCYP1A1; h1A1/hGSTP1-25, V79MZh1A1 cell line expressing stably transfected hGSTP1, clone 25; h1A1/hGSTP1-23, V79MZh1A1 cell line expressing stably transfected hGSTP1, clone 23 (cell line with the highest GST activity).

Figure 2. Comparison of B[a]P metabolite profiles produced by human versus rat CYP1A1 expressed in V79MZ cells

Transgenic cell lines expressing stably transfected human (V79MZh1A1) or rat (V79MZr1A1) CYP1A1 were plated, exposed to B[a]P, and the medium harvested and analyzed by reverse-phase HPLC. Results of a single experiment are expressed as a percent of the total metabolites detected with each cell line. Data are from Table 5 of Jacob, et al. Polycyclic Aromatic Compounds 10: 1–9, 1996 (ref. 28); used with permission, Taylor & Francis, Ltd., <http://www.informaworld.com>.

Figure 3. Effect of GSH depletion on protection by GSTs against B[a]P

Cytotoxicity assays were performed as described in Materials and Methods. Glutathione depletion was induced in one set of plates by addition of BSO eight hours after plating. The preincubation with BSO was for 16 hr prior to B[a]P addition and continued for the duration of the B[a]P exposure. Open bars, no BSO; gray bars, 5 µM BSO; black bars, 10 µM BSO. Abbreviations: V79MZ, non-transfected parental control cell line; V79MZh1A1, V79MZ cell line expressing only stably transfected hCYP1A1; h1A1/hGSTP1-23, V79MZh1A1 cell line expressing stably transfected hGSTP1, clone 23 (cell line with the highest GST activity). Results are the mean $\pm$ S.D. of three or more experiments; only the cells expressing hGSTP1 and treated with 10 µM BSO differed significantly (* $p < .01$) from the untreated control culture.

Table I

Activities of GST isozymes and CYP1A1 in transgenic V79 cell lines. Results are the mean $\pm$ S.D. of 3 or more independent assays.

Cell line	GST (nmol/min/mg)	CYP1A1 (pmol/min/mg)
Human CYP1A1		
V79MZ (control)	209 $\pm$ 54	0.5 $\pm$ 0.2
V79MZh1A1	140 $\pm$ 52 ¹	19.7 $\pm$ 3.1 ⁶
V79MZh1A1/hGSTP1-25	992 $\pm$ 131 ²	17.8 $\pm$ 4.8 ^{4, 6}
V79MZh1A1/hGSTP1-23	3181 $\pm$ 429	14.9 $\pm$ 4.3 ^{4, 6}
Rat CYP1A1		
V79MZr1A1	107 $\pm$ 23 ^{3, 4}	10.8 $\pm$ 2.9
V79MZr1A1/hGSTP1-33	2538 $\pm$ 164 ⁵	10.1 $\pm$ 1.1 ⁷

¹ p $\geq$ 0.2, relative to V79MZ

² p < .002, relative to V79MZh1A1/hGSTP1-23

³ p < .05, relative to V79MZ

⁴ p $\geq$ 0.2, relative to V79MZh1A1

⁵ p > .05, relative to V79MZh1A1/hGSTP1-23

⁶ p < .005, relative to V79MZ

⁷ p > 0.5, relative to V79MZr1A1

Table II

Protection against B[a]P cytotoxicity by transfected hGSTP1 in transgenic V79 cells expressing hCYP1A1 or rCYP1A1. Fold-resistance is the ratio of the IC₅₀ of B[a]P in the GST-transfected cell lines to the IC₅₀ of the V79MZh1A1 or V79MZr1A1 control cell lines. Results are the mean $\pm$ S.D. of four or more independent assays.

Cell Line	BaP IC ₅₀ (μ M)	Fold-Resistance
Human CYP1A1		
V79MZ (control)	7.5 $\pm$ 1.0	
V79MZh1A1	0.27 $\pm$ 0.15	1.0
V79MZh1A1/hGSTP1-25	1.6 $\pm$ 0.8 ¹	5.9
V79MZh1A1/hGSTP1-23	4.2 $\pm$ 0.6 ²	16
Rat CYP1A1		
V79MZr1A1	2.4 $\pm$ 0.7	1.0
V79MZr1A1/hGSTP1-33	6.0 $\pm$ 1.5 ¹	2.5

¹ p < 0.01 in comparison with the V79MZh1A1 or V79MZr1A1 cell line.

² p < 0.001 in comparison with the V79MZh1A1 cell line.

Table III

Protection against DB[a,l]P cytotoxicity by transfected hGSTP1 in transgenic V79 cells expressing hCYP1A1 or rCYP1A1. Fold-resistance is the ratio of the IC₅₀ of DB[a,l]P in the GST-transfected cell lines to the IC₅₀ of the V79MZh1A1 or V79MZr1A1 control cell lines. Results are the mean $\pm$ S.D. of three independent assays.

Cell Line	DB[a,l]P IC ₅₀ (μ M)	Fold-Resistance
Human CYP1A1		
V79MZ (control)	> 1000	
V79MZh1A1	2.9 $\pm$ 1.3	1.0
V79MZh1A1/hGSTP1-23	20 $\pm$ 2.4 ^I	7.0
Rat CYP1A1		
V79MZr1A1	33 $\pm$ 3.1	1.0
V79MZr1A1/hGSTP1-33	48 $\pm$ 7.6	1.5

^I p < 0.001 in comparison with the V79MZh1A1 cell line.

Table IV

Protection against cytotoxicity of the pure (+) – or (–)-B[a]P-7,8-dihydrodiol enantiomers by transfected hGSTP1 in transgenic V79 cells expressing hCYP1A1 or rCYP1A1. Fold-resistance is the ratio of the IC₅₀ of B[a]P in the GST-transfected cell lines to the IC₅₀ of the V79MZh1A1 or V79MZr1A1 control cell lines. Results are the mean ± S.D. of three independent assays.

Cell Line	(+)-BaP-Diol IC ₅₀ (nM)	Fold- Resistance	(-)-BaP-Diol IC ₅₀ (nM)	Fold- Resistance
Human CYP1A1				
V79MZ (control)	> 1000		> 1000	
V79MZh1A1	8.5 ± 2.6	1.0	8.5 ± 3.2	1.0
h1A1/hGSTP1-23	42 ± 0.6 ¹	5.0	20 ± 4.7 ³	2.4
Rat CYP1A1				
V79MZr1A1	8.3 ± 1.3	1.0	8.0 ± 0.5	1.0
r1A1/hGSTP1-33	17 ± 1.5 ²	2.0	12 ± 3.2 ⁴	1.5

¹ p < 0.0001;

² p = 0.002;

³ p = 0.02;

⁴ not significant, p > 0.1 compared to the matched parental cell line expressing only hCYP1A1 or rCYP1A1.

Table V

Protection against cytotoxicity of racemic DB[a,l]P-11,12-dihydrodiol by transfected hGSTP1 in transgenic V79 cells expressing hCYP1A1 or rCYP1A1. Fold-resistance is the ratio of the IC₅₀ for the GST-transfected cell lines to the IC₅₀ for the V79MZh1A1 or V79MZr1A1 control cell lines. Results are the mean ± S.D. of three independent assays.

Cell Line	DB[a,l]P - 11,12-diol IC ₅₀ (μM)	Fold-Resistance
Human CYP1A1		
V79MZ (control)	93 ± 8.2	
V79MZh1A1	0.37 ± 0.24	1.0
V79MZh1A1/hGSTP1-23	3.4 ± 0.9 ^I	9.2
Rat CYP1A1		
V79MZr1A1	0.85 ± 0.36	1.0
V79MZr1A1/hGSTP1-33	1.6 ± 0.8	1.9

^I p < 0.01 in comparison with the V79MZh1A1 cell line.