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Overexpression of Glutathione S-Transferase π Enhances the Adduct Formation of Cisplatin with Glutathione in Human Cancer Cells

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In this paper, we provide direct evidence that glutathione S-transferase π (GSTπ) detoxifies cisplatin (CDDP). We used human colonic cancer HCT8 cells sensitive and resistant to CDDP, the level of cisplatin–glutathione adduct (DDP–GSH) being higher in the resistant cells. There was an overexpression of GSTπ mRNA in these CDDP-resistant cells. Incubation of the cells with CDDP resulted in the formation of DDP–GSH dependent on the CDDP concentration and the incubation time. The formation of DDP–GSH was abolished when the cells were pre-treated with ethacrynic acid or ketoprofen, inhibitors of GSTπ. Purified GSTπ also catalyzed the formation of DDP–GSH *in vitro*, with an apparent K_m of 0.23 mM for CDDP and an apparent V_{max} of 4.9 nmol/min/mg protein. The increase in DDP–GSH produced by GSTπ was linear with incubation time up to 3 h and optimal of pH 7.4. A GSTπ transfectant cell line was constructed in HCT8 cells using a pcDNA3.1 (−)/Myc-His B with an expression vector containing cDNA for GSTπ. Transfection of GSTπ cDNA into HCT8 cells resulted in an increase in the expression of GSTπ by 1.4-fold in parallel with an augmentation of the formation of DDP–GSH. These results suggest that GSTπ plays a role in the formation of DDP–GSH and the acquisition of resistance to CDDP in cancer cells.

Keywords: Glutathione S-transferase π, cisplatin, adduct

Abbreviations: GSH, reduced form of glutathione; GST, glutathione S-transferase; GPX, glutathione peroxidase; γ-GCS, γ-glutamylcysteine synthetase; GR, glutathione reductase; CDDP, cisplatin [*cis*-diamminedichloroplatinium(II)]; DDP–GSH, cisplatin–glutathione adduct; SDS-PAGE, SDS-polyacrylamide gel electrophoresis

INTRODUCTION

Cisplatin (*cis*-diamminedichloroplatinum(II), CDDP) is an anti-cancer drug containing platinum and widely used to treat solid tumors, such as small cell lung cancer, ovarian cancer, colonic cancer, and leukemia. The development of resistance to CDDP is a major concern and is often associated with an increase in the intracellular levels of glutathione (GSH).[1] CDDP cytotoxicity

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increases when intracellular GSH is decreased, whereas resistance to CDDP is associated with a marked increase in the GSH synthesis.[2] GSH functions to protect mammalian cells against the effects of radiation, oxidative damage, and certain toxic compounds of endogenous and exogenous origins.[3,4] These functions are important to the acquisition of drug-resistance to CDDP, nitrogen mustard, etc.[5]

Glutathione S-transferase (GST, EC 2.5.1.18) is ubiquitous in nature. GST functions in xenobiotic biotransformation, drug metabolism, and protection against peroxidative stress.[6,7] In most mammalian cells, the enzyme is soluble or membrane-bound and forms conjugates of GSH with electrophiles and plays a role in determining the sensitivity of cells to toxic and carcinogenic agents. GSTπ is one of a family of GST. GSTπ has been shown to be overexpressed in various human cancer tissues made resistant to chemotherapeutic drugs.[8]

Saburi *et al.*[9] reported an increase in the levels of GSTπ isozyme in Chinese hamster ovary cell lines resistant to CDDP. Induction of GSTπ in these cells enhanced the resistance to CDDP.[10] Doroshow *et al.*[11] reported that transduction of GSTπ induced multi-drug resistance. These results suggest that GSTπ is involved in the acquisition of CDDP-resistance. Furthermore, an important role for GSTπ in chemical carcinogenesis has been suggested by experiments using GSTπ transfectant cell lines.[12]

Our laboratory previously found that the transport activity of cisplatin–glutathione adduct (DDP–GSH) is augmented in CDDP-resistant cancer cells.[1] We also found that the activity of GSTπ increased in these CDDP-resistant cells. However, the exact role of GSTπ in the acquisition of drug-resistance has not been well clarified. In this report, we studied the role of GSTπ in the detoxification of CDDP in CDDP-resistant cancer cells and the formation DDP–GSH using purified GSTπ. The effect of the overexpression of GSTπ on the formation of DDP–GSH was also studied.

MATERIALS AND METHODS

Materials

CDDP was a gift from Nihon Kayaku Co. (Tokyo, Japan). Chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO). RPMI 1640 and FBS were from Gibco (Grand Island, NY). [^{3}H]L-Glycine, [α-^{32}P]dCTP, [^{3}H]GSH, and [^{125}I]protein A were from New England Nuclear (Boston, MA). CDDP-resistant cells were donated by Dr. K.J. Scanlon (Berlex Biosciences, CA).

Preparation of Cells

We used human colonic cancer cells sensitive (HCT8) and resistant (HCT8DDP) to CDDP and human ovarian cancer cells sensitive (A2780) and resistant (2780DDP) to CDDP. Cancer cells were maintained in RPMI 1640 supplemented with 10% FBS at 37°C in 5% CO_2 with 100% humidity. About 2×10^6 cells were harvested by centrifugation at 4°C. The cytosolic fraction was lysed and recovered by adding four volumes of 10 mM NaH_2PO_4/Na_2HPO_4 (pH 7.4) containing 0.5 mM EDTA, 0.1 mM 2-mercaptoethanol, and 0.5 mM phenylmethylsulfonyl fluoride, followed by sonication for 2 min. The sensitivity of these cells to CDDP was assayed using a tetrazolium salt.[1] Cells (5000–10,000) were placed in 150 µl of medium per well in 96-well plates. Twenty-four hours after various concentrations of CDDP were added, the cells were used for the cytotoxicity assay. The cytotoxicity is expressed as the IC_{50}.

Enzyme Assay

The activity of glutathione peroxidase (GPX, EC 1.11.1.9), glutathione reductase (GR, EC 1.6.4.2) and GST was estimated as described.[13] One unit of enzyme activity is expressed as 1 µmol substrate changed per min. GSTπ was purified from human placenta according to the method described by Guthenberg *et al.*[14] A specific antibody was prepared as described by Shiratori

et al.[15] The specific immunoadsorbent method was employed for the estimation of the specific activity of GSTπ as described.[16] An affinity column of Sepharose 4B coupled with anti-human GSTπ rabbit IgG (10 mg IgG/ml of Sepharose 4B) was used for this estimation.

Estimation of Glutathione

The concentration of GSH was estimated by means of enzyme recycling as described by Beutler.[13]

Immunological Estimation

Immunological levels of GSTπ in the cells were estimated by Western blotting. Lysate (5 μg) from the cell extracts was separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) in a 15% gel, transferred to a nitrocellulose membrane, and immunologically stained using rabbit anti-human GSTπ.[17] To measure the relative immunological activity, the membranes were incubated with [^{125}I]protein A (0.2 mCi/ml) for 1 h, extensively washed, autoradiographed, and analyzed using a Fujix Bio-Analyzer BAS-2000 (Fuji Photo Film, Tokyo, Japan). The relative radioactivity is expressed as photostimulated luminescence (PSL).[18] The protein concentration was determined according to Redinbaugh and Turley,[19] with BSA as the standard.

Extraction of Cellular RNA and Northern Blot Analysis

The human GSTπ probe was constructed as described.[20] Total RNA was isolated using the RNeasy Total RNA kit (Qiagen, Hilden, Germany), and the concentrations of all RNA samples were determined spectrophotometrically at 260 nm. RNA samples were resolved by electrophoresis in 1% agarose gels containing 0.6 M formaldehyde, transferred to nylon membranes, and then hybridized with ^{32}P-labeled nick-translated probes for GSTπ. The membranes were autoradiographed and analyzed using a Fujix Bio-Analyzer BAS-2000. The relative radioactivity is expressed as PSL. Levels of 28S rRNA were determined using a ^{32}P-labeled synthetic oligonucleotide.

Formation of DDP–GSH

The formation of DDP–GSH was studied using HPLC essentially as described.[1] Cells were incubated with CDDP for a certain period, then the incubation mixture was treated with 5% trichloroacetic acid and centrifuged at 15,000 rpm for 5 min. An aliquot of the supernatant was drawn and subjected to HPLC for the estimation of DDP–GSH. The effect of GSTπ on the formation of DDP–GSH was estimated using purified GSTπ. Two and five to ten units of GSTπ were incubated with 2.2 mM CDDP and 4.4 mM GSH in 150 μl of phosphate buffered saline (9 parts 0.154 M NaCl and 1 part 0.1 M NaH_2PO_4/Na_2HPO_4, pH 7.4) in the presence or absence of inhibitors for 1 h at 37°C. The trichloroacetic acid-extract of the assay mixture was subjected to HPLC for the estimation of DDP–GSH formed. Assay mixture in the absence of GSTπ was used as a background. For the kinetic study, 10 units of GSTπ, various concentrations of CDDP and 4.4 mM GSH were employed.

Transport of DDP–GSH

The transport activity of DDP–GSH was estimated essentially as described.[1] Briefly, the cells were incubated with 50 μM CDDP for 1 h at 37°C, then washed three times with 3 ml of the incubation buffer and resuspended in the medium buffer for 30 min before being centrifuged at 1000× *g* for 5 min. The supernatant was analyzed by HPLC.

Transfection of GSTπ cDNA

The GSTπ cDNA was prepared by a RT-PCR method using total RNA extracted from HCT8

cells as a template. The product of RT-PCR was inserted into a PCRII vector (Invitrogen, San Diego, CA) and transfected to JM109 competent cells. Partial digestion of the GSTπ cDNA (630 bp) was achieved using XhoI and EcoRI. The expression vector for the GSTπ cDNA was constructed using a plasmid, pcDNA 3.1 (−)/Myc-His B (Invitrogen) containing a SV40 promoter, and the GSTπ cDNA. Transfection of the expression vector for the GSTπ cDNA was performed by a lipofection method using a transfection reagent (Promega, Madison, MI). HCT8 cells transfected with an expression vector not containing the insert of GSTπ cDNA were used as a control. Stable transfectants were obtained by step-wise addition of G418 (GIBCO) to the incubation medium at 150 μg/ml to 900 μg/ml over a one-month period.

Statistical Analysis

The data are given as the mean ± SD. Differences were calculated with Student's *t* test.

RESULTS

Levels of GSTπ in CDDP-resistant Cells

The activity of GST was 3.7 times higher in HCT8DDP than in HCT8, and 1.7 times higher in A2780DDP than in A2780. A concomitant increase in the immunological activity of GSTπ (Figure 1A and B) and the expression of GSTπ mRNA (Figure 1C and D) was observed in these CDDP-resistant cells. The immunological levels of GSTπ were 3.8 times higher in HCT8DDP than HCT8, and 1.9 times higher in A2780DDP than A2780 ($p < 0.01$). The expression of GSTπ mRNA in HCT8DDP was 3.6 times higher and that in A2780DDP was 1.5 times higher than that in control cells ($p < 0.01$) respectively. These results indicate that the increase in the activity of GST in CDDP-resistant cells is due to the elevation of the expression of GSTπ mRNA.

In Vivo Study

To further study the metabolism of CDDP and antioxidants inside the cells, HCT8DDP cells were incubated with 50 μM CDDP for 1 h, and the supernatant was applied to a HPLC column. The formation of DDP–GSH depended on the concentration of CDDP in the range 25–100 μM. The levels of DDP–GSH after treating the cells for 1 h with 25 μM CDDP were 18 ± 2 pmol/10^6 cells, 35 μM CDDP were 26 ± 3 pmol/10^6 cells, 50 μM CDDP were 39 ± 5 pmol/10^6 cells, and 100 μM CDDP were 75 ± 9 pmol/10^6 cells (Table I). This formation was inhibited when the cells were incubated with CDDP in the presence of ethacrynic acid or ketoprofen, inhibitors of GSTπ.[21,22]

In Vitro Study

It has been reported that incubating CDDP with GSH results in the formation of DDP–GSH at a molar ratio of one to two.[1,18] To study the physiological role of GSTπ, 2.2 mM CDDP was incubated with a two molar excess of GSH in the presence of 10, 5 and 2.5 units of purified human GSTπ (68 units/mg protein) for 1 h at 37°C, and the incubation mixture was applied to a HPLC column. As shown in Table II, DDP–GSH formed. DDP–GSH adduct was prepared using a HPLC column. The effect of platinum was determined by an atomic absorption method and that of GSH by an amino acid analysis. The levels of DDP–GSH depended on the concentration of GST: 22.5 nmol/150 μl at 10 units of GSTπ, 11.0 nmol/150 μl at 5 units, and 5.5 nmol/150 μl at 2.5 units. Ethacrynic acid and ketoprofen inhibited the formation of DDP–GSH, suggesting that GSTπ helps to form DDP–GSH as a transferase. Table III shows the results of the time course study on the formation of DDP–GSH by GSTπ. When 2.2 mM CDDP and 4.4 mM GSH were incubated without GSTπ, the non-enzymatic formation of DDP–GSH was linear up to 4 h. Addition of 10 units of GSTπ further increased

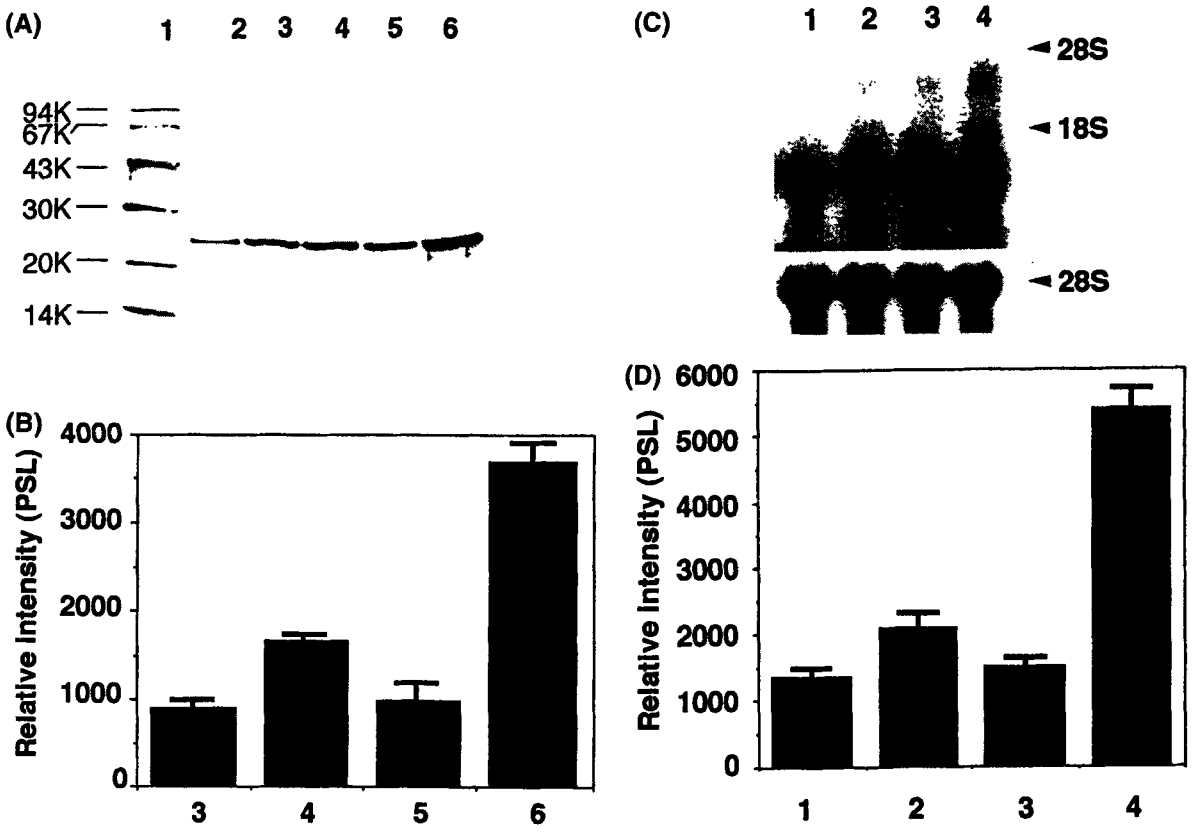

FIGURE 1 Expression of GSTπ. (A) Immunological staining of GSTπ was evaluated by Western blotting. Proteins separated by SDS-PAGE in a 15% gel were transferred to a nitrocellulose membrane and immunologically stained using anti-GSTπ rabbit IgG. Lane 2, purified GSTπ; Lane 3, A2780 cells; Lane 4, A2780DDP cells; Lane 5, HCT8 cells; Lane 6, HCT8DDP cells. Molecular weight standards (Lane 1) are as noted. (B) Each lane corresponds to the cell lines as described in A. (C) Northern blots of the GSTπ mRNA. About 30 μg of each RNA extracted from the cells was fractionated by electrophoresis through 1% agarose, transferred to nylon membranes, and then hybridized with ^{32}P-labeled nick-translated GSTπ DNA. Lane 1, A2780 cells; Lane 2, A2780DDP cells; Lane 3, HCT8 cells; Lane 4, HCT8DDP cells; Bottom, levels of 28S rRNA. (D) Each lane corresponds to that in Lane C. The relative amounts of the radioactivity are expressed as PSL. Data are the means of three independent analyses; bars, SD.

the DDP–GSH formation dependent on the incubation time. Figure 2 shows the results of a kinetic study of GSTπ. Various concentrations of CDDP were incubated with 4.4 mM GSH and 10 units of GSTπ and the formation of DDP–GSH was estimated. The apparent K_m value of GSTπ was 0.23 mM for CDDP with a V_{max} value of 4.9 nmol/min/mg protein. The addition of GST prepared from human hepatocytes to the incubation mixture did not result in the formation of DDP–GSH (data not shown).

Overexpression of GSTπ

The effect of the transfection of GSTπ cDNA on the formation of DDP–GSH was studied. We constructed a GSTπ transfectant cell line. Transfection of GSTπ cDNA into HCT8 cells (HCT8G12 cells) resulted in an augmentation of the expression of GSTπ mRNA by 1.4-fold on Northern blot analysis (Figure 3). The GST activity in HCT8G12 cells increased concomitantly. There was no apparent change in the levels of GSH and the activity of

TABLE I Effect of various concentrations of CDDP on the formation of DDP–GSH

CDDP concentration (μM)	Inhibitor		Intracellular DDP–GSH formed (pmol/10^6 cells)
	Ethacrynic acid (μM)	Ketoprofen (μM)	
25	—	—	18 ± 2 (A)
35	—	—	26 ± 3*
50	—	—	39 ± 5* (B)
100	—	—	75 ± 9*
50	50	—	13 ± 2**
50	—	50	15 ± 4**

HCT8DDP cells were treated with 25–100 μM CDDP for 1 h and the formation of DDP–GSH was estimated by HPLC as described in Materials and Methods. The cells had been pretreated with 50 μM ethacrynic acid or 50 μM ketoprofen 1 h and incubated with 50 μM CDDP for 1 h. The effect of inhibitors for GSTπ on the formation of DDP–GSH was estimated. Values refer to the amount of DDP–GSH formed (pmol/10^6 cells) and are the mean ± SD for three independent analyses.
*$p < 0.05$ vs. (A). **$p < 0.05$ vs. (B).

TABLE II Effect of GSTπ inhibitors

GSTπ (units)	Inhibitor		DDP–GSH formed (nmol)
	Ethacrynic acid (mM)	Ketoprofen (mM)	
2.5	—	—	5.5 ± 2.0 (A)
5	—	—	11.0 ± 4.0*
10	—	—	22.5 ± 5.0* (B)
10	2.0	—	1.2 ± 0.5**
10	—	2.0	3.0 ± 1.0**

The effect of ethacrynic acid and ketoprofen on the formation of DDP–GSH was examined *in vitro*. CDDP (2.2 mM) was mixed with 4.4 mM GSH in the presence of various concentrations of GSTπ for 1 h. Two mM ethacrynic acid or 2 mM ketoprofen was added to the incubation medium in the presence or absence of 10 units of GSTπ in 150 μl of assay mixture. Values are the mean ± SD for three independent analyses.
*$p < 0.05$ vs. (A). **$p < 0.05$ vs. (B).

TABLE III Time course study

Incubation time (h)	DDP–GSH formed (nmol)	
	GSTπ (−)	GSTπ (+)
1	19.0 ± 2.0	41.5 ± 3.0
2	41.5 ± 5.0	82.9 ± 5.0
3	58.0 ± 6.0	121.0 ± 7.0
4	77.5 ± 5.0	145.0 ± 7.0

The effect of incubation time on the formation of DDP–GSH was examined *in vitro* using HPLC. The incubation mixture comprised of 2.2 mM CDDP and 4.4 mM GSH was incubated in the presence or absence of 10 units of GSTπ in 150 μl of assay mixture. Values are the mean ± SD for three independent analyses.

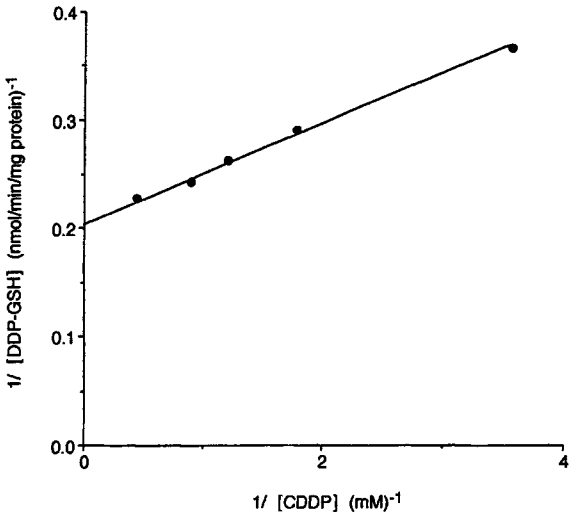

FIGURE 2 A Lineweaver–Burk plot of the formation of DDP–GSH. Effect of GSTπ on the formation of DDP–GSH was estimated using purified GSTπ. Briefly, 10 units of GSTπ was incubated with various concentrations of CDDP and 4.4 mM GSH in a 150 μl of phosphate buffured saline (9 parts of 0.154 M NaCl and 1 part of 0.1 M NaH_2PO_4/Na_2HPO_4 pH 7.4) for 1 h at 37°C. The trichloroacetic acid-extract of the assay mixture was applied to HPLC for the estimation of DDP–GSH formed. Assay mixture in the absence of GSTπ was used as a background.

GSH-related enzymes, such as GPX, GR, SOD (Cu, Zn-SOD and Mn-SOD) in the transfected cells (Table IV). Table V shows the results of the estimation of DDP–GSH formation. The levels of DDP–GSH formed were 26 ± 3 pmol/10^6 cells in HCT8G12 cells and 19 ± 2 pmol/10^6 cells in the control cells (HCT8V2). It was found that the augmentation of the formation of DDP–GSH in HCT8G12 cells paralleled the increase in the expression of GSTπ. However, there was no difference in the transport activity of DDP–GSH and the cell viability against CDDP.

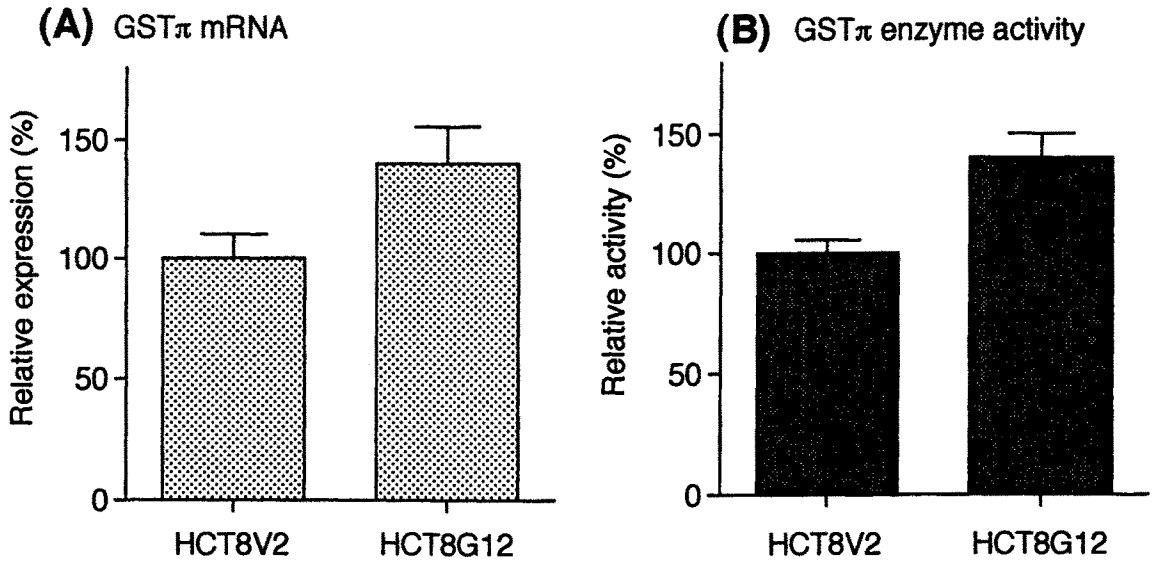

FIGURE 3 Expression of GSTπ in the GSTπ-transfected cells. The mRNA expression and the activity of GSTπ was estimated in GSTπ-transfected HCT8G12 cells and the control HCT8V2 cells. (A) Northen blot analysis. (B) The activity of GST. It was expressed as a percentage of that in HCT8V2 cells. Data are the means of three independent analyses; bars, SD.

TABLE IV Concentration of GSH and activities of its related enzymes

Cell line	GSH (nmol/10^6 cells)	GST (milliunits/10^6 cells)	GPX (milliunits/10^6 cells)	GR (milliunits/10^6 cells)	Total SOD (milliunits/10^6 cells)
HCT8	20.0 ± 1.6	27 ± 1.3	7.5 ± 0.4	21.0 ± 1.1 (20.0 ± 0.5)	54.9 ± 3.8
HCT8V2	22.3 ± 1.9	28 ± 1.7	6.0 ± 0.2	20.0 ± 2.3 (19.0 ± 2.5)	63.5 ± 4.3
HCT8G12	23.8 ± 2.5	39 ± 2.2	4.5 ± 0.4	19.5 ± 1.2 (18.9 ± 2.2)	62.6 ± 3.5

Values are the mean ± SD for three independent experiments. The activity of GR was estimated in the presence or absence (parentheses) of FAD.

TABLE V Formation of DDP–GSH

Cells	DDP–GSH		Cell viability IC_{50} (μM)
	Intracellular formed (pmol/10^6 cells)	Transport activity (pmol/10^6 cells/min)	
HCT8V2	19 ± 2	2.3 ± 0.3	52 ± 4
HCT8G12	26 ± 3	2.1 ± 0.2	57 ± 6

The control HCT8V2 cells and GSTπ transfected HCT8G12 cells were treated with 50 μM CDDP for 1 h and the formation of DDP–GSH inside the cells and the transport activity was evaluated by HPLC. Cell viability was estimated by MTT assay in the presence of various concentration of CDDP. Values are the mean ± SD for three independent analyses.

DISCUSSION

Studies on the acquisition of resistance to CDDP have indicated a correlation to several processes, such as the influx of CDDP inside the cells, the binding of CDDP to DNA to cause damage, detoxification through the coupling of CDDP with GSH to form adduct, and the efflux of DDP–GSH outside the cells.[24]

GSH is synthesized in most mammalian cells through ATP-requiring steps catalyzed by γ-glutamylcysteine synthetase (γ-GCS, EC 6.3.2.2) and glutathione synthetase. γ-GCS catalyzes the rate-limiting step of GSH synthesis.[25] As reported previously, resistance to CDDP in cancer cells was accompanied by increases in the levels of GSH and the activities of γ-GCS, and GST.[1] In this study we found that almost all the activity of GST in the cancer cells was dependent on that of GSTπ. The increase in the genetic and immunological levels of GSTπ were greater in the CDDP-resistant cells than the control cells (Figure 1). Induction of GSTπ by CDDP has been reported,[9] and the properties of CDDP-resistant variants of chinese hamster ovary cells compared. Miyazaki *et al.*[10] found a correlation between the CDDP-resistance and the expression of GSTπ. Transfection of the GSTπ gene to these cells also caused CDDP-resistance. Doroshow *et al.*[11] provided evidence that the simultaneous overexpression of GSTπ and *MDR*1, multi-drug resistance 1, produced broad-range multi-drug resistance including CDDP-resistance. Nakagawa *et al.*[17] have reported that GSTπ transcfectant cell lines showed resistance to adriamycin but increased levels of GSTπ *per se* were not sufficient to convey resistance to CDDP. These results suggest that GSTπ functions together with other processes are required for the CDDP-resistance. An effect of the transfection of GSTπ antisense cDNA on the sensitivity to CDDP was reported by Ban *et al.*[26] Our results are consistent with reports that elevation of GSTπ correlates with CDDP-resistance.

Previously we found that the formation of intracellular DDP–GSH is augmented 2-fold in HCT8DDP cells compared to in HCT8 cells.[1] In the present study, the significance of GSTπ in the formation of DDP–GSH was studied using HCT8DDP cells. The synthesis of DDP–GSH in the cells decreased when the cells were pre-treated with inhibitors for GSTπ, ketoprofen or ethacrynic acid (Table I). The effect of ketoprofen and ethacrynic acid on GSTπ has been reported.[21,22] These inhibitors inhibit only GSTπ and not other GST isozymes. We next obtained direct evidence that GSTπ catalyzes the formation of DDP–GSH using GSTπ purified from human placenta (Tables II and III). The addition of GSTπ to the assay mixture containing GSH and CDDP resulted in an increase in the formation of DDP–GSH compared to that without GSTπ, even though the apparent V_{max} value of this enzyme for DDP–GSH formation is not very high (Figure 2). In contrast, GSTs prepared from human liver had no effect on the formation of DDP–GSH. This suggests that GSTπ only possesses affinity for CDDP.

A non-enzymatic formation of the complex CDDP/GSH was previously reported,[23] in a study using a laser mass-spectrometer. The apparent molecular weight was approximately 809, corresponding to the molecular weight of a CDDP-GSH complex with a 1/2 molar ratio. In the present study, we found an enzymatic formation of DDP–GSH using GSTπ with the incubation time of 1 h, while the previously reported formation was non-enzymatic and took longer. In this study, no difference in the peak of DDP–GSH in the presence or absence of GSTπ was detected by HPLC (data not shown).

GSTπ cDNA transfected into HCT8 cells (Table IV) and the overexpression of GSTπ caused an augmentation of DDP–GSH (Table V). This strongly suggests that GSTπ catalyzes the formation of DDP–GSH. We expected the increase in the formation of DDP–GSH by the overexpression of GSTπ to cause an increase in the DDP–GSH transport and the cell viability. However, there was no apparent increase in the transport activity of DDP–GSH nor the cell viability against CDDP in the GSTπ transfected cells (Table V). The reason for this is not clear at present. Overexpression of GSTπ may have some influence on the expression of the DDP–GSH transporters.

Chester *et al.*[27] showed that the activity of GSTπ, elevated in drug-resistant cancer cells, correlates with the inhibition of DNA cross-link formation. The regulatory elements of the 5′

flanking region of GSTπ have been clarified.[28,29] Moffat *et al.*[30] showed that the induction of GSTπ is mediated by AP-1 in drug-resistant cancer cells. One of the mechanisms by which induction of GSTπ is brought about is the activation of AP-1 by oxygen radicals, whose binding site is located on the *GSTπ* gene.[29] Oxygen radical species produced in CDDP-exposed cells may cause induction of GSTπ. GSTπ catalyzes the reactions of both the formation of DDP–GSH and the peroxidation of GSH, suggesting that GSTπ plays an important role in the detoxification of CDDP and the acquisition of resistance to CDDP in cancer cells.

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