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ORIGINAL ARTICLE

Mechanistic study of potentiation of chemotherapy by a haloenol lactone derivative in vitro

Wei Wang · Cindy Q. Xia · Ning Liu · Liang-Shang Gan · Jiang Zheng

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Abstract
Purpose The objective of this study was to understand the biochemical mechanisms by which a haloenol lactone (HEL) derivative potentiates cisplatin-induced cytotoxicity in vitro. HEL was originally designed and synthesized as a site-directed inactivator of glutathione *S*-transferase π isozyme (GST-π). Over-expression of GST-π has been found to be associated with chemotherapy resistance.
Methods A concentration-dependent GST inhibition was assessed after UOK130 cells were exposed to HEL at concentrations of 10 and 20 μM. Potentiated cytotoxicity was evaluated by treatment of UOK130 cells with a selection of alkylating agents in the presence or absence of HEL. Intracellular glutathione (GSH) was determined after exposure to HEL. Protective effect of GSH was examined by co-treatment with GSH ester in UOK130 cells exposed with a combination of cisplatin and HEL. Multiple resistance-associated protein (MRP) 1–3 activity was assayed by determining the rate of ^{3}H-LTC$_4$ and ^{3}H-E$_2$17βG through the MRPs into recombinant membrane vesicles.
Results Exposure of HEL at 10 and 20 μM caused 28 and 41% of inhibition of cellular GST activity. Cytotoxicity of cisplatin, chlorambucil, and melphalan was enhanced 1.8–2.7-fold by HEL at 10 μM. No significant protection effect by GSH ester exposure was observed on cisplatin toxicity co-treated with HEL. HEL was found to inhibit MRP1, MRP2, and MRP3 with IC$_{50}$ of 1.30, 28.2, and 3.66 μM, respectively.
Conclusion Haloenol lactone showed inhibitory effect on GST-π and MRP1-3 (selective inhibition of MRP1 and MRP3), and it was also found to deplete intracellular GSH.

Keywords Haloenol lactone · GST-π selective inhibitor · MRP inhibition · Potentiation in drug resistance

W. Wang · J. Zheng
Department of Pharmaceutical Sciences,
Northeastern University, Boston, MA 02115, USA

C. Q. Xia · N. Liu
Drug Metabolism and Pharmacokinetics,
Millennium Pharmaceuticals Inc,
Cambridge, MA 02139, USA

L.-S. Gan
Drug Discovery Support, Boehringer-Ingelheim
Pharmaceuticals, Inc, Ridgefield, CT 06877, USA

J. Zheng (✉)
Center for Developmental Therapeutics,
Seattle Children's Hospital Research Institute,
Division of Gastroenterology, Department of Pediatrics,
University of Washington, 1900 9th Avenue,
Seattle, WA 98101, USA
e-mail: jiang.zheng@seattlechildrens.org

Introduction

Innate or acquired resistance in chemotherapy remains a serious impediment toward the use of drugs in the treatment of cancer, especially for the alkylating agents. Although the etiology of drug resistance is multifactorial, the overexpression of drug detoxification factors, such as P-glycoprotein and glutathione *S*-transferase (GST), remain the most common alterations underlying drug resistance in laboratory models [4].

Glutathione *S*-transferases are a family of enzymes that play a critical role in the protection of cells from carcinogenic and cytotoxic xenobiotics by catalyzing the addition of GSH to electrophiles. A variety of anticancer drugs, such as chlorambucil and cisplatin, have been demonstrated to be direct substrates of GSTs [4]. Numerous

resistant cell lines have been shown to express high levels of GSTs by chronic exposure to anticancer agents. These enzymes are believed to be responsible for the metabolism (detoxification) of chemotherapeutics in cancer treatment. It has become evident that overexpression of GST isozymes (particularly the π isozyme) plays a significant role in acquired drug resistance of tumor cells [11].

The established role of GSTs in drug metabolism and overexpression of these enzymes in a wide range of tumor tissues provided a rationale for the development of GST inhibitors to circumvent the resistance phenotype. The first generation of GST inhibitors included ethacrynic acid and had promising activity in vitro. Unfortunately, lack of isozyme specificity and side effects limited its clinical use [10]. Because GSTs are an important avenue for the detoxification of alkylating agents in normal cells, any effective way of inhibition of these enzymes as an adjuvant for alkylating agents' chemotherapy must take into consideration the possible deleterious effects on the defenses of normal cells. Isozyme specific inhibitors are likely to cause fewer problems in this regard [8].

A haloenol lactone (HEL) derivative (Fig. 1), an α-bromoketone precursor, was synthesized as an inactivator of GST-π [15]. This compound was designed to take advantage of the presence of multiple potentially reactive nucleophiles in the active site of GST-π. Those nucleophiles near the active site of GST-π could facilitate the opening of the lactone ring. The ring opening leads to the formation of an α-bromoketone intermediate (Fig. 1) capable of irreversible modification of the protein with isozyme selectivity based on the reactivity of residues near the active site [6]. This HEL can potentially be used as a synergetic agent for cancer chemotherapy, particularly for the GST-π overexpressed drug-resistant cancers [13, 16].

In this present work, we demonstrate that HEL (at non-toxic concentration) potentiated cytotoxicity induced by alkylating agents in UOK130 cells. The potentiation of HEL resulted from both GST inhibition and GSH depletion. HEL was also found to inhibit GSH conjugates (GS-X) efflux pumps: multiple resistance-associated protein 1 (MRP1), MRP2 and MRP3.

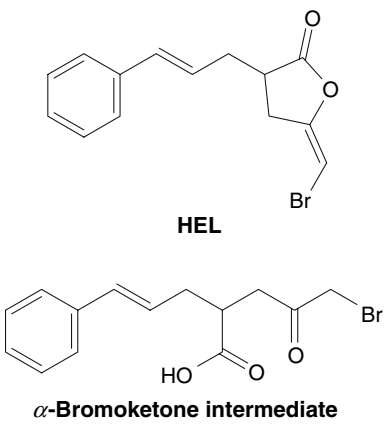

Fig. 1 Structure of the haloenol lactone (*HEL*) and α-bromoketone intermediate

Materials and methods

Chemicals

HEL (3-Cinnamyl-5(*E*)-bromomethylidenetetrahydro-2-furanone) (Fig. 1) was synthesized as reported in our earlier paper [15]. Cisplatin, chlorambucil, melphalan, glutathione (GSH), GSH ester, and 1-chloro-2, 4-dinitrobenzene (CDNB) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). MK571, ^{3}H-leukotriene C_4 (^{3}H-LTC_4), and ^{3}H-estradiol-17-β-D-glucuronide (^{3}H-$E_2$17βG) were prepared at Millennium Pharmaceuticals Inc. (Cambridge, MA, USA).

Cell culture

UOK130, a human renal carcinoma cell line, was obtained from Dr. Michael DeGregorio's lab at UC Davis Cancer Center. Cell culture media and supplies were purchased from Mediatech Inc. (Herndon, VA, USA). The serum for cell culture was from Perbio Hyclone Co. (Logan, UT, USA). UOK130 cells were grown as monolayer culture in improved minimum essential medium (IMEM) supplemented with 10% fetal bovine serum and 100 U/ml penicillin/streptomycin (Invitrogen, Carlesbad, CA, USA). Cells were held in an incubator chamber containing humidified 95% air and 5% CO_2 at 37°C. All cells were used in the log-phase of growth unless otherwise specified.

Cytotoxicity assay

Cisplatin, chlorambucil, melphalan, HEL, GSH ester, individually or in combination, were evaluated for their cytotoxic effects on cell growth in vitro under specified conditions. The final percentage of organic solvents (ethanol or DMSO) for dissolving the chemicals in cell culture medium was less than 0.5%. In vitro cytotoxicity was assessed using the cell proliferation reagent WST-1 (Roche Diagnostics, Indianapolis, IN, USA). Briefly, after a 24-h incubation with anticancer drugs, the WST-1 reagent (10 μl/well) was added directly to the medium, and cells were incubated for 2 h. The absorbance was measured at 450 nm wavelength by Versa Max UV-visible spectrometer (Molecular Devices, Sunnyvale, CA, USA).

GST activity assay

Cells were then harvested, washed with ice-cold PBS buffer, and sonicated. The cell homogenates were centrifuged at 14,000*g* for 30 min at 4°C. The supernatants were collected for analysis of GST activity according to the method of Habig [5]. The reaction mixture contained 100 mM potassium phosphate buffer, pH 6.5, 1 mM GSH, 1 mM CDNB. The reaction was started by the addition of GST samples prepared as described above. The rate of product formation of the CDNB conjugate with GSH was determined spectrophotometrically by monitoring the absorbance at 340 nm in a 6-min kinetic model (read per 30 s), using a VERSA Max UV-visible spectrophotometer. Specific activities were based on protein concentrations as determined using BCA protein assay kit from Pierce Inc. (Rockford, IL, USA).

Cellular GSH content assay

Intracellular GSH content was measured by ApoGSH^{TM} Glutathione Kit (BioVision Research Products, Mountain View, CA, USA). Briefly, cells were harvested by trypsin-EDTA and counted. Cells (1×10^6) were collected and then centrifuged at 700*g* for 5 min. The cell pellets were washed with 1 ml ice-cold PBS buffer, followed by centrifugation. The cell pellets were re-suspended in 100 µl ice-cold cell lysis buffer, incubated on ice for 10 min, and then centrifuged at 14,000*g* for 10 min at 4°C. The supernatants were transferred to a 96-well plate. To each well, 2 µl of 25-mM monochlorobimane (MCB) and 2 µl of the 50-U/ml GST reagent were added. The plate was incubated at 37°C for 30 min and the fluorescence at Ex/Em = 380/460 nm was measured with a Gemini fluorescence photometer (Molecular Device, Sunnyvale, CA, USA).

Membrane vesicle transport assay

Multiple resistance-associated protein 1 (MRP1), MRP2 and MRP3 membrane vesicles purified from recombinant baculovirus-transduced Sf9 cells were purchased from Solvo Biotech Co. (Gyár, Hungary). The interaction is detected as the modulation of the initial rate of ^{3}H-LTC$_4$ (MRP1 and MRP2), ^{3}H-E$_2$17βG (MRP3) transport by MRPs into membrane vesicle purified from Sf9 cells expressing the human transporters. Briefly, 10 µl membrane vesicle was incubated with 1 µM ^{3}H-LTC$_4$ or ^{3}H-E$_2$17βG, 2 mM GSH and testing compound in 0.1 M MOPS-Tris buffer. The experiment started with 20 µl 0.2 M MgATP or 0.1 M MgAMP and incubated at 37°C shaking plate for 4 min (^{3}H-LTC$_4$) or 15 min (^{3}H-E$_2$17βG). After incubation, the vesicles were washed to a filter plate. The filter plate was dried at 70°C for 2 h. Then 50 µl scintillation cocktail was added to a filter plate and the radioactivity was measured in a liquid scintillation spectrophotometer (Beckman, Fullerton, CA, USA). ATP-dependent transport (cpm) was calculated by subtracting cpm values measured in the presence of AMP from the cpm values measured in the presence of ATP for the control and samples. To find the ATP-dependent transport (%): calculate the percent inhibition of the test compound, using the following formula:

$$\frac{\text{ATP dependent transport in the presence of test drug (cpm)}}{\text{ATP dependent transport in drug free control (cpm)}} \times 100\%$$

Data analysis

Data were expressed as Mean ± SD. Determination of possible synergism [combination index (CI)] was calculated by the Chou–Talalay equation [2, 3]. CI < 1, CI = 1, CI > 1 indicate synergism, additive effect and antagonism, respectively. Analysis of variance (ANOVA) was applied to evaluate the muitlple group data. A value of $P < 0.05$ was accepted as a statistically significant level. Curve fittings and graph were performed using XLfit 4.0 (IDBS, Surrey, UK) and Sigmaplot 9.0 (SYSTAT, Point Richmond, CA, USA).

Results

As an initial step, we identified a concentration at which HEL acts as a reasonably non-toxic modulator, which is useful in situations where conventional chemotherapeutic agents are detoxified by GST-π [4]. In vitro cytotoxicity studies of HEL in UOK130 cells showed the LD$_{10}$ of HEL is about 20 µM. Our previous studies indicated that HEL at 10 µM inhibited about 28% of cytosolic GST activity and 41% of GST activity at 20 µM, when it was incubated with UOK130 cells [13]. The optimized concentration of HEL for potentiation studies was chosen as 10 µM in UOK130 cells.

As shown in Table 1, the cytotoxicity of cisplatin was potentiated by HEL (at 10 µM) co-incubation in UOK130 cell, with an IC$_{50}$ shift from 28 µM (cisplatin alone) to 10 µM (co-incubation). This drug combination showed synergism, and the combination index (CI) at IC$_{50}$ was about 0.71.

The potentiation by HEL was also observed in UOK130 cells after exposure to chlorambucil and melphalan. The IC$_{50}$ shifts were found from 50 to 27 µM for chlorambucil treatment and from 44 to 21 µM for melphalan treatment

Table 1 Potentiation of cytotoxicity of chemotherapeutic agents by HEL in UOK130 cells

Chemotherapeutic agents	IC_{50} of cisplatin cytotoxicity (mean ± SD, μM, $n = 6$)		Resistant factor	Combination index (CI) at IC_{50}
	Drug alone	Drug + HEL (10 μM)		
Cisplatin	28.03 ± 5.39	10.53 ± 3.17	2.7	0.710
Chlorambucil	50.37 ± 2.28	27.62 ± 3.05	1.8	0.859
Melphalan	44.54 ± 5.22	21.22 ± 4.59	2.1	0.763

(Table 1). Drug combination studies demonstrated significant synergism when HEL was combined with these anticancer drugs (Table 1). These results suggest that HEL may act as a chemotherapeutic sensitizer in vitro.

The observed potentiation by HEL mentioned above led us to investigation of the underlying biochemical mechanisms by which HEL enhanced the cytotoxicity by chemotherapeutic agents. We continued using the UOK130 cell line as an experimental model. Our earlier studies indicated that the cells selectively expressed GST-π, which allowed us to investigate the role of a single GST isozyme in detoxification of alkylating agents [15]. A concentration-dependent inhibition of GST activity by HEL was reported by this laboratory [13].

In addition to GST activity, intracellular concentration of GSH, a co-substrate of GSH conjugation, is critical for the detoxification of alkylating agents. We compared intracellular GSH levels in UOK130 cells after exposure to cisplatin in the presence or absence of HEL. As shown in Fig. 2, a significantly decreased GSH levels were observed in the cells co-treated with HEL relative to that of cisplatin-treatment-alone group. The depletion of intracellular GSH by HEL cannot be excluded as a factor in the potentialtion of cisplatin toxicity by HEL, since GSH conjugation depends not only on GST activity but also on intracellular GSH contents.

To determine the role of GSH depletion in HEL-induced potenciation of cisplatin toxicity, we examined whether compensation of the depleted GSH by HEL could reverse the potenctiation of cisplatin toxicity. We determined the intracellular GSH contents after UOK130 cells were treated with HEL alone. As shown in Fig. 3a, treatment of HEL at 10 μM caused 20% drop in intracellular GSH level comparative to that of the control group. The ED_{50} for GSH depletion of HEL is about 100 μM (data not shown). In a parallel study, we incorporated GSH ester in the exposure system. As expected, the depleted GSH by HEL (at 10 μM) was restored by co-incubation with GSH ester (at 0.5 mM) in UOK130 cells (Fig. 3a). Previous study from this laboratory demonstrated that HEL could be conjugated with GSH spontaneously, and the resulting GSH-HEL conjugate was identified by LC-MS [14]. We believe that HEL may spontaneously deplete intracellular GSH. However, we are unable to exclude the possible involvement of GST-π, although some of the enzyme was inactivated by HEL.

To differentiate the role of GST inactivation from that of GSH depletion in HEL potentiation, we examined the protective effect of GSH ester on cisplatin toxicity potentiated by HEL through exposure of UOK130 cells to cisplatin along with HEL in the presence and absence of GSH ester. The presence of GSH ester did not show the protective effect, and a significant potentiation by HEL was observed with an IC_{50} shift from 27.4 to 13.7 μM and a calculated combination index (CI) of 0.825 (Fig. 3b), suggesting the synergism of the drug combination. Hence, this indicates that the inhibition upon GST-π by HEL plays a major role in HEL potentiation of cisplatin cytotoxicity to UOK130 cells.

Multiple resistance-associated proteins (MRP1, MRP2 and MRP3) have been recognized as GSH conjugate (GS-X) efflux pumps on cell membrane, and over-expression of the pumps is considered to be another biochemical mechanism

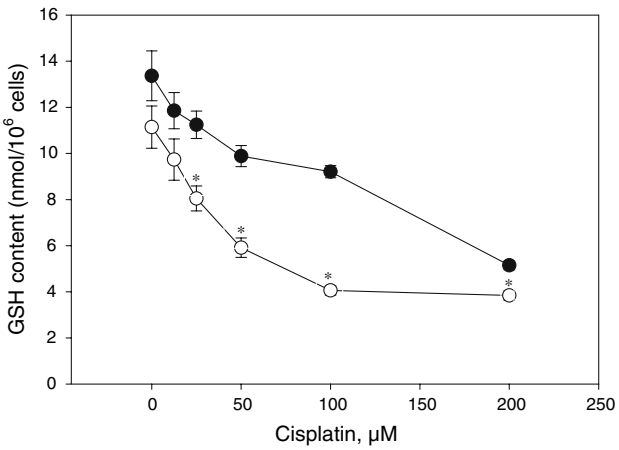

Fig. 2 GSH depletion in UOK130. UOK 130 cells were plated in six-well plates (1×10^6 cells/well/2 ml of medium) and incubated with HEL at 37 °C overnight. Cisplatin was then added to wells at various concentrations and incubated for 24 h. After incubation, cells were harvested by trypsinization and dissolved in cell lysis buffer. The cell lysates were centrifuged for 30 min and the supernatants were collected for GSH assay by use of ApoGSH™ Glutathione kit. Fluorescence values were measured at 380/460 nm. GSH content was expressed as nmol GSH/10^6 cells. Data are mean ± SD of three determinations. (*filled circle* cisplatin alone; *open circle* cisplatin + HEL (10 μM). *Compared with the cisplatin alone group, $P < 0.05$

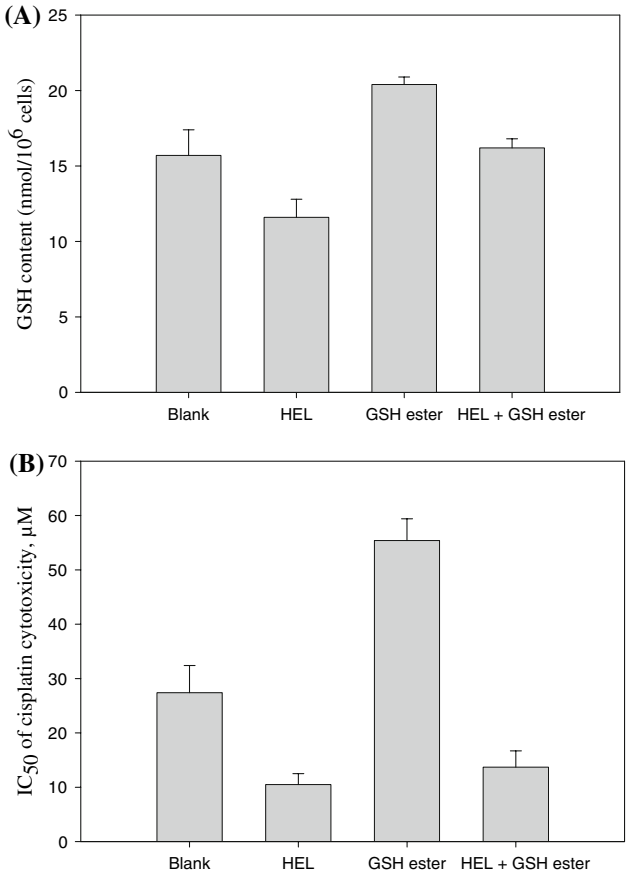

Fig. 3 **a** Intracellular GSH level in UOK130 cells after exposure to GSH modulators: after incubation with HEL (10 μM) or GSHe (0.5 mM) for 24 h, cells were harvested by trypsinization and dissolved in cell lysis buffer. The cell lysates were centrifuged for 30 min and the supernatants were collected for GSH measurement using ApoGSH^{TM} Glutathione kit. Fluorescence values were measured at 380/460 nm. GSH content was expressed as nmol GSH/10^6 cells. Data are mean ± SD of three determinations. **b** Cytotoxicity of cisplatin in UOK130 cells after exposure to GSH modulators: cells were treated with HEL (10 μM) or GSHe (0.5 mM) for 4 h; cisplatin was then added to wells at various concentrations and incubated for 24 h. After incubation, WST-1 reagent (10 μl/well) was added directly to the medium, and cells were incubated for 2 h. The absorbance was measured at 450-nm wavelength. Data represent the averages of three different experiments; *bars* SD

of acquired chemotherapy resistance [14]. Previous studies reported that some GST inhibitors might also inhibit the GS-X efflux pump on the membrane. For example, TLK-117, another GST-π selective inhibitor, increased the accumulation of daunorubicin by some inhibitory effect on MRP1 [9]. Cisplatin is well known to be a substrate of GSH conjugation to form GS-X. Naturally, we cannot rule out the role of potential MRP inhibition resulting in accumulation of cisplatin conjugates in cells after exposure to HEL. We examined the expression of MRP1, MRP2 or MRP3 in UOK130 cells by Western blot, but we did not find the existence of MRP1-3 (data not shown).

Table 2 IC_{50} of HEL and MK571 in transport of report compound through MRPs

MRPs	IC_{50} of HEL (μM)	IC_{50} of MK571 (μM)
MRP1	1.30 ± 1.12	0.56 ± 0.21
MRP2	28.22 ± 7.55	5.06 ± 1.07
MRP3	3.66 ± 1.48	1.83 ± 0.70

The inhibitory effect of HEL and MK571 was detected as the modulation of the initial rate of ^{3}H- LTC_4 and ^{3}H-$E_2$17βG through MRP transporters into membrane vesicles purified from recombinant baculovirus-transduced Sf9 cells. Data are expressed as mean ± S.D. (n = 3)

Recombinant MRP1, MRP2 and MRP3 membrane vesicles purified from baculovirus-transduced Sf9 cells are widely accepted as experimental tools for various MRP studies. The vesicular transport assay is based on the closed membrane vesicles that are in an "inside-out" orientation in the membrane preparations. Some efflux transporters carry substrates into the vesicles from the buffer in which the membranes are suspended. Rapid filtration of the membrane suspension allows separation of substrate molecules that have been transported into the membrane vesicles from molecules that have not, and the amount of transported substrate can be detected using suitable detection methods. The standard vesicular transport assay provides information on any interaction between the ABC transporter, and the test drug that would affect the transport of the reporter compounds (^{3}H-LTC_4 for MRP1 and MRP2, ^{3}H-$E_2$17βG for MRP3). As shown in Table 2, HEL demonstrated the inhibitory effect on MRP1-3. Compared with MK571, a known MRP inhibitor, HEL showed the similar inhibitory potency but better selectivity upon MRP1 and MRP3.

Discussion

GSTs have emerged as promising therapeutic targets because specific isozymes are overexpressed in a wide variety of tumors and may play a role in the etiology of other diseases, including neurodegenerative diseases, multiple sclerosis and asthma [10]. A variety of GST inhibitors were shown to reverse drug resistance by potentiating cytotoxicity of anticancer drugs.

Efforts have been made to develop a GST inhibitor with more optimal isozyme specificity [8]. Our previous studies demonstrated that HEL potentiated the toxicity of a selection of widely used anticancer drugs in tumor cells [13]. The present biochemical investigation suggested that GST-π inhibition might not be the only factor responsible for HEL potentiation. Instead, combining HEL with chemotherapeutic drugs resulted in the following: (a) isozyme-selective

GST-π inhibition to reduce drug detoxification; (b) mild GSH depletion to intensify the potentiation effect of HEL; (c) inhibition upon GS-X efflux pumps to cause intracellular accumulation of GS-X.

Interestingly, tumor cells express high levels of GST-π and tend to over-express the isozyme, no matter whether they have been exposed to anticancer drugs [7]. This offers the possibility of selective GST-π inhibition as a means to decrease the side effects and increase the chemotherapeutic index of anticancer agents. It is predicted that HEL causes irreversible inactivation of GST-π in tumor but the selective enzyme inhibition may not hurt normal tissues much, due to specific tissue distribution of GST-π [11]. Enzyme GST and co-substrate GSH are two factors important to GSH conjugation reactions. Either inhibition of GST or depletion of intracellular GSH may lead to profound detoxification of alkylating agents for chemotherapy. We believe that HEL is a mild GSH depletor. The mild GSH depletion by HEL may not result in severe adverse effect, because the physiological intracellular GSH concentration is relatively high (up to 10 mM) in normal tissues [12]. However, the GSH depletion together with GST inactivation by HEL in tumor tissues may work cooperatively to further inhibit the GST-mediated drug detoxification and reverse drug resistance.

Another important property of HEL, worth noting, is its inhibitory selectivity upon MRP1 and MRP3. MRP1, MRP2 and MRP3 are three very similar organic anion membrane transporters. They have similar trans-membrane structures, physiological functions, substrate specificity and tissue distribution [1]. According to our knowledge, HEL is one of a few compounds to show selective inhibitory effect upon MRP1 and MRP3. The mechanism of selective inhibition upon MRP1 and MRP3 is still to be determined. Our results suggested that HEL might be considered to be used as a MRP1 or MRP3-selective inhibitor in future transporter studies.

In summary, HEL potentiates cisplatin cytotoxicity not only by the inhibition of GST-π but also by depletion of GSH. HEL was also was found to selectively inhibit MRP1 and MRP3.

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References

1. Borst P, Evers R, Kool M, Wijnholds J (2000) A family of drug transporters: the multidrug resistance-associated proteins. J Natl Cancer Inst 92:1295–1302
2. Chou TC, Motzer RJ, Tong Y, Bosl GJ (1994) Computerized quantitation of synergism and antagonism of taxol, topotecan, and cisplatin against human teratocarcinoma cell growth: a rational approach to clinical protocol design. J Natl Cancer Inst 86:1517–1524
3. Chou TC, Talalay P (1984) Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul 22:27–55
4. Gate L, Tew KD (2001) Glutathione *S*-transferases as emerging therapeutic targets. Expert Opin Ther Targets 5:477–489
5. Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione *S*-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 249:7130–7139
6. Mitchell AE, Zheng J, Hammock BD, Lo Bello M, Jones AD (1998) Structural and functional consequences of haloenol lactone inactivation of murine and human glutathione S-transferase. Biochemistry 37:6752–6759
7. Montali JA, Wheatley JB, Schmidt DE (1995) Comparison of glutathione *S*-transferases in levels in predicting the efficacy of a novel alkylating agent. Cell Pharm 2:241–247
8. Morgan AS, Ciaccio PJ, Tew KD, Kauvar LM (1996) Isozyme-specific glutathione S-transferase inhibitors potentiate drug sensitivity in cultured human tumor cell lines. Cancer Chemother Pharmacol 37:363–370
9. O'Brien ML, Vulevic B, Freer S, Boyd J, Shen H, Tew KD (1999) Glutathione peptidomimetic drug modulator of multidrug resistance-associated protein. J Pharmacol Exp Ther 291:1348–1355
10. Tew KD, Ronai Z (1999) GST function in drug and stress response. Drug Resist Updat 2:143–147
11. Townsend DM, Tew KD (2003) The role of glutathione-*S*-transferase in anti-cancer drug resistance. Oncogene 22:7369–7375
12. Townsend DM, Tew KD, Tapiero H (2003) The importance of glutathione in human disease. Biomed Pharmacother 57:145–155
13. Wang W, Liu G, Zheng J (2007) Human renal UOK130 tumor cells: a drug resistant cell line with highly selective over-expression of glutathione *S*-transferase-pi isozyme. Eur J Pharmacol 568:61–7
14. Zheng J, Liu G, Tozkoparan B, Wen D (2005) Mechanistic studies of inactivation of glutathione *S*-transferase Pi isozyme by a haloenol lactone derivative. Med Chem 1:191–198
15. Zheng J, Mitchell AE, Jones AD, Hammock BD (1996) Haloenol lactone is a new isozyme-selective and active site-directed inactivator of glutathione *S*-transferase. J Biol Chem 271:20421–20425
16. Zheng J, Wurz GT, Cadman TB, Degregorio MW, Jones AD, Hammock BD (1997) Haloenol lactone: a new synergist of chemotherapy in vitro. Biochem Biophys Res Commun 241:13–17