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Reversal of anticancer drug resistance by COTC based on intracellular glutathione and glyoxalase I

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Abstract—Suppression of resistance to anticancer drugs by COTC of glyoxalase I (GloI) inhibitor targeting intracellular glutathione (GSH) and GloI was studied. Depletion of the cellular GSH content and inhibition of GloI by COTC increased chemotherapy-mediated apoptosis in apoptosis-resistant pancreatic adenocarcinoma AsPC-1 cells.

Studies of the resistance of tumor cells to cytotoxic drugs are necessary for understanding of the mechanisms of the cells' defense against injury. Investigation of the insensitivity of malignant cells to chemotherapy is needed also in oncology practice because drug resistance is often considered to be a cause of tumor therapy failures. Ineffectiveness of the therapy may be provoked by many other causes besides tumor cell alterations. The most widely investigated mechanisms with known clinical significance are (1) activation of transmembrane proteins effluxing different chemical substances from the cells (P-glycoprotein is the most well-known pump);[1] (2) alterations of the genes and the proteins involved in the control of apoptosis (especially p53 and Bcl-2);[2] (3) activation of the glutathione detoxification system.[3] The cellular glutathione system is a critical component of detoxification of cytostatics in the cell. Glutathione (GSH), a non-protein thiol, can interact via its thiol with the reactive site of a drug, resulting in conjugation of the drug with GSH.[4]

Furthermore, increased levels of GSH have been found in cell lines resistant to alkylating agents (e.g., cisplatin (CDDP), chlorambucil, melphalan, cyclophosphamide).[5] The glyoxalase system is composed of two enzymes, glyoxalase I (GloI, EC4.4.1.5), and glyoxalase II (GloII, EC3.1.2.6), and GSH as an essential cofactor.[6] GloI catalyses isomerization of the hemiacetal, formed spontaneously from α-oxoaldehyde (RCOCHO) and GSH, into *S*-2-hydroxyacylglutathione derivatives. GloII catalyses the conversion of *S*-2-hydroxyacylglutathione derivatives into α-hydroxyacids and re-forms GSH consumed in the GloI-catalyzed reaction step (Fig. 1).

It is believed that a major physiological role for this enzyme system is as a detoxification pathway for cytotoxic methylglyoxal (MG),[7] and this substrate accumulates markedly when GloI is inhibited in situ by cell-permeable GloI inhibitors and by depletion of GSH.[8] GloI is frequently overexpressed in malignant tissues and tumor cell lines compared with corresponding normal levels. Hence, potent GloI inhibitors could prove to be valuable therapeutic agents and biological probes.[9]

In 1975 Umezawa and co-workers isolated a new inhibitor of GloI having structure **1** from the culture broth of *Streptomyces griseosporeus*.[10] Known as COTC, compound **1** showed strong inhibition of the growth of

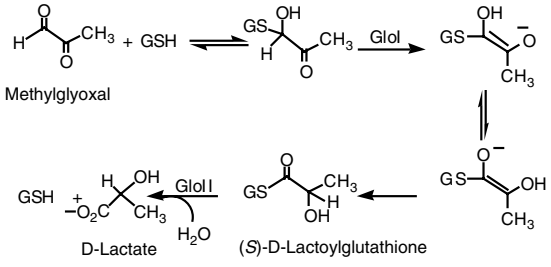

Figure 1. Metabolism of methylglyoxal by the glyoxalase system.

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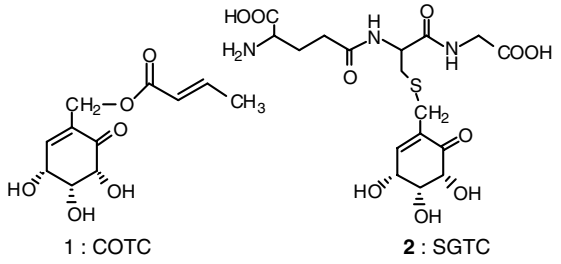
HeLa cells and inhibition of Ehrlich ascites carcinoma by daily injection. The crotonyloxy group of **1** shows a surprising proclivity to be displaced by SH-compounds (e.g., glutathione, cysteine, 2-mercaptoethanol, or thiophenol).[11] This property is shown to be the basis for its biological activity. Recently, the structure of the active GloI inhibitor from COTC has been conclusively identified by means of total synthesis as compound SGTC (COTC–GSH conjugate) and human GloI is competitively inhibited by SGTC ($K_i = 183 \pm 6$ μM) but is not inhibited by COTC itself in the absence of GSH.[12]



In the continuous research to find new suppressors of anticancer drug resistance targeting GSH and GloI, we studied apoptosis induction by COTC in apoptosis-resistant human pancreatic adenocarcinoma cells (AsPC-1).[13] At first, we estimated the intracellular GSH content of AsPC-1 cells.[14] As shown in Table 1, the content of GSH was detected in AsPC-1 cells at higher levels than in apoptosis-sensitive pancreatic tumor cell line BxPC-3 and fibrosarcoma cell line HT1080. This finding suggested that the increased level of GSH would be one factor leading to the apoptotic resistant cells.

Table 1. Intracellular GSH contents

Cell line	GSH (nmol/mg protein)
AsPC-1 (apoptosis resistant)	352.5 ± 128.2
BxPC-3 (apoptosis sensitive)	58.5 ± 23.0
HT 1080	18.9 ± 6.5

AsPC-1: human pancreatic adenocarcinoma cell.
BxPC-3: human pancreatic tumor cell.
HT-1080: human fibrosarcoma cell.

Next, we examined the effects of COTC on cellular GSH content of AsPC-1 cells. The GSH level declined rapidly after addition of 30 μg/mL of COTC, which bottomed at 40% of the initial level of GSH at 1–2 h and a normal level of GSH was recovered 12 h after the addition of COTC and, furthermore, a dose-dependent decrease of the GSH level in the cells was observed by treatment of COTC for 1 h at between 0.1 and 100 μg/mL (Fig. 2).[15]

Then, we examined the conjugation reaction of COTC with [^{35}S]-GSH by a method of autoradiography in the coexistence of melphalan because it is known that tumor

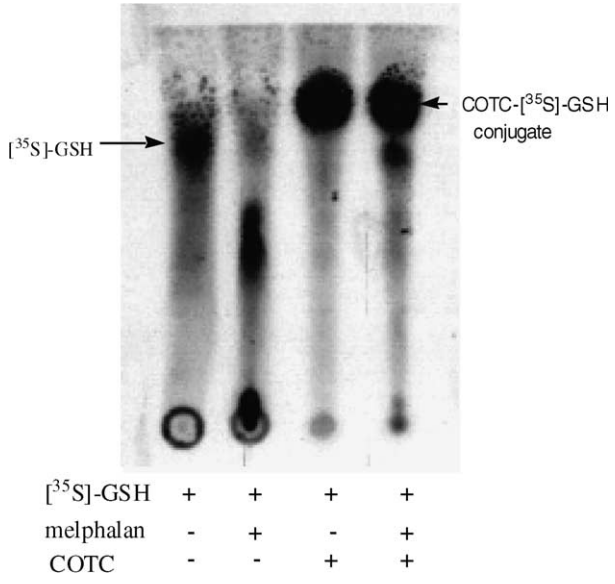


Figure 3. Conjugation reaction of GSH with COTC and melphalan. [^{35}S]-GSH (2 μCi) and/or melphalan (10 mM) and/or COTC (10 mM) were mixed for 6 h. Then, samples were developed with MeOH/aq NaCl (1:6) on RP-C18 TLC plate and detected by autoradiography.

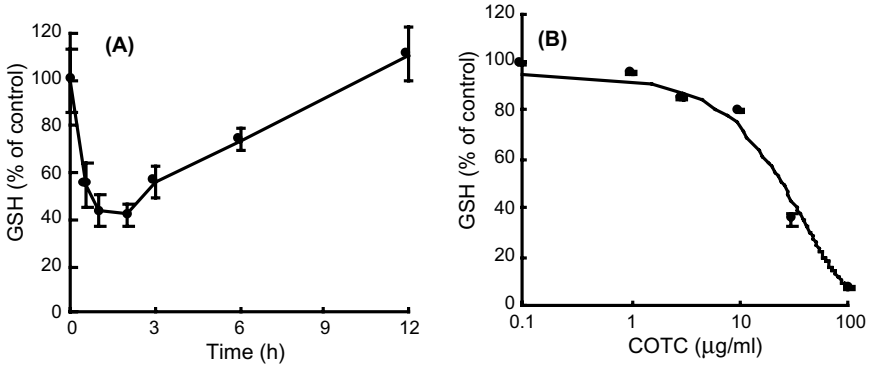


Figure 2. Effect of COTC on GSH level in AsPC-1 cells. (A) Cells were treated with 30 μg/mL COTC for the indicated periods. Then, cells were lysed and assayed for GSH. Values are the means ± SD of quadruplicate determinations. (B) Cells were treated with various concentrations of COTC for 1 h. Then cells were lysed and assayed for GSH. Values are mean ± SD of quadruplicate determinations.

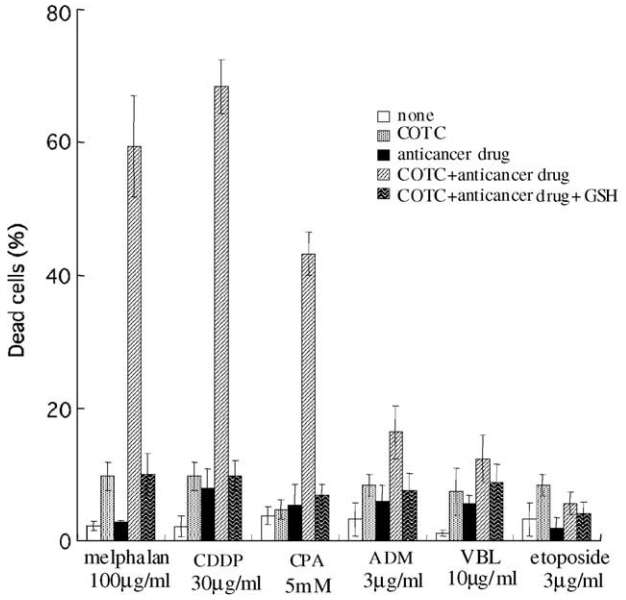


Figure 4. Effect of anticancer drugs on the cell viability of COTC-treated AsPC-1 cells. Cells were treated with anticancer drugs in the absence or presence of 30 mg/mL COTC and/or 1 mM GSH for 24 h. Cell viability was determined by trypan blue dye exclusion assay. Values are mean ± SD of quadruplicate determinations.

cells use GSH mainly as a drug-detoxifying system for this alkylating agent through conjugation of the cytostatic with GSH. Interestingly, as shown in Figure 3, the formation of COTC–GSH conjugate preferentially and selectively occurred compared with the formation of melphalan–GSH conjugate. This observation suggests that COTC can react with GSH much faster than alkylating agents react with GSH.

On the basis of such preliminary data described above, we evaluated the effect of COTC on the suppression of resistance to anticancer drugs of AsPC-1 cells. In accordance with prediction, alkylating agents such as melphalan, CDDP, and cyclophosphamide (CPA) increased their cell death potency remarkably in the presence of COTC, and this aspect of increased effect was completely cancelled when added GSH simultaneously. COTC did not potentiate significantly the cell death of adriamycin (ADM), vinblastin (VBL), and etoposide (Fig. 4). Next, we tested dose effect of melphalan and COTC on the viability of AsPC-1 cells. As shown in Figure 5, the capability of cell death of melphalan was induced by COTC in the manner of dose dependence. Furthermore, to elucidate whether the induced cell death is apoptotic, detections of DNA fragmentation by melphalan and CDDP in COTC-treated AsPC-1 cells were performed by the Tunel stainings[16] and we found that COTC plays an active role in inducing the apoptotic process in AsPC-1 cells (Fig. 6).

In summary, we conclude from our study that the GSH content is comparatively high in apoptosis-resistant pancreatic adenocarcinoma AsPC-1 cells and GSH plays an important role in resistance to chemotherapy of alkylating anticancer drugs. The combined inhibitory effects of COTC on GSH and GloI may lead to raised intracellular concentrations of anticancer drugs and toxic 2-oxoaldehydes. Consequently, depletion of GSH and inhibition of GloI by COTC increase chemotherapy-mediated apoptosis, which may lead to new curative and palliative strategies against pancreatic carcinoma.

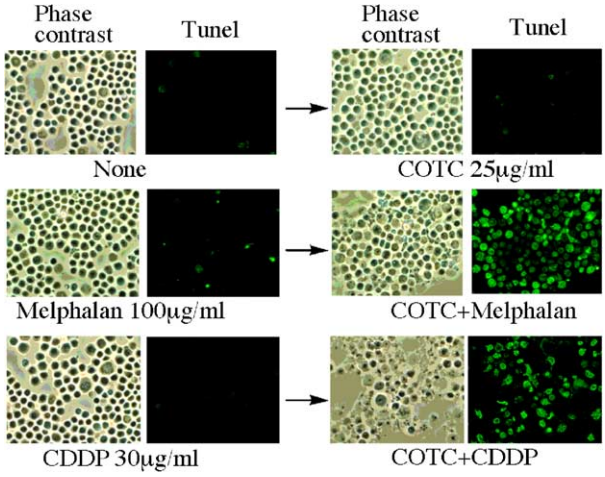


Figure 6. DNA fragmentation by melphalan and CDDP in COTC-treated AsPC-1 cells. Cells were treated with 100 μg/mL melphalan and 30 μg/mL CDDP in the absence or in the presence of 25 μg/mL COTC. Then the cells were fixed and stained with Tunel.

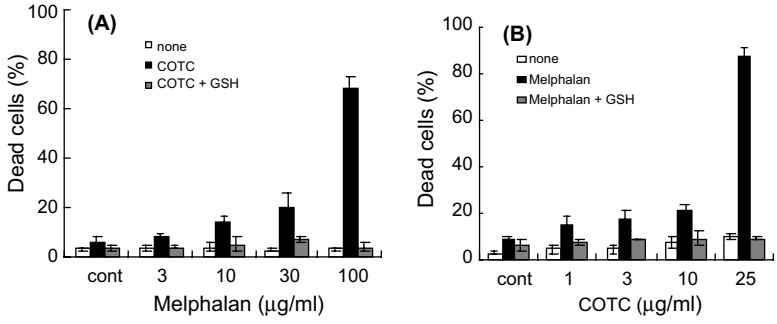


Figure 5. Dose effect of melphalan and COTC on the viability of AsPC-1 cells. (A) Cells were treated with various concentration of melphalan in the absence or presence of 30 μg/mL COTC and/or 1 mM GSH for 24 h. (B) Cells were treated with various concentration of COTC in the absence or presence of 100 μg/mL melphalan and/or 1 mM GSH for 24 h. Cell viability was determined by trypan blue dye exclusion assay. Values are mean ± SD of quadruplicate determinations.

Our results also suggest that a GloI inhibitor could be an effective chemotherapeutic agent against tumor cells showing drug-resistance because of the overexpression of GSH and GloI.

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