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Comparison of glutathione S-transferase activity between drug-resistant and -sensitive human tumor cells: Is glutathione S-transferase associated with multidrug resistance?

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Summary. We have studied the levels of glutathione S-transferase in drug-resistant and -sensitive human tumor cell lines to examine a possible involvement of glutathione S-transferase (GST) in multidrug resistance mechanisms. No increase in the activity of glutathione S-transferase was detected in myelogenous leukemia K562 resistant to adriamycin (K562/ADM), ovarian carcinoma cell line A2780 resistant to adriamycin (2780AD), or acute lymphoblastic leukemia cell line CCRF-CEM resistant to vinblastine (CEM-VLB100), compared with the drug-sensitive parent tumor cells. The human breast cancer cell lines Hattori and MCF-7 had a 12- to 63-fold lower level of glutathione S-transferase activity than K562, A2780, CCRF-CEM, and their drug-resistant sublines. Induction of ADM resistance in Hattori did not increase the activity of glutathione S-transferase. However, induction of colchicine resistance in MCF-7 resulted in a 70-fold increase in the activity of glutathione S-transferase. A revertant of the colchicine-resistant MCF-7 contained a level of glutathione S-transferase activity similar to that of the resistant subline. The increase of glutathione S-transferase activity did not alter the sensitivity of the cell to cytotoxic drugs. The increased activity was due to the appearance of glutathione S-transferase π, as shown by enzyme inhibition using anti-glutathione S-transferase π antibody. Our findings indicate that increased cellular glutathione S-transferase activity is not associated with the development of multidrug resistance.

Introduction

One of the serious obstacles to chemotherapy is the development of drug-resistant tumor cells. Resistance to antitumor agents such as anthracyclines and vinca alkaloids is associated with a variety of phenotypic alterations. The elevated expression of a plasma membrane component termed P-glycoprotein is associated with multidrug resistance [6, 8, 9, 11]. In addition, alterations in cytosolic levels of enzymes or components are associated with multidrug resistance. Batist et al. [1] have reported that resistance in an adriamycin-resistant human breast cancer subline was associated with a 45-fold increase in glutathione S-transferase activity (GST). This increased activity was due to the appearance of an isoenzyme that is immunologically related to GST activity in human placenta. GST represents a family of isoenzymes that catalyze the conjugation of glutathione to a wide variety of electrophilic substances, and it plays a physiological role in the detoxification of xenobiotics in vivo [3, 4]. We have examined the level of GST in several drug-resistant and -sensitive cell lines to elucidate a possible correlation between glutathione S-transferase and multidrug resistance. In this report we show that the induction of multidrug resistance is not associated with an increase in GST activity.

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Abbreviations: GST, glutathione S-transferase; ADM, adriamycin; VCR, vincristine; VLB, vinblastine

Materials and methods

Chemicals. Reagent-grade glutathione was obtained from Kojin Co. Ltd., Tokyo, Japan, and 1-chloro-2,4-dinitrobenzene was the product of Tokyo Kasei Co. Ltd., Tokyo, Japan. The antitumor agents were obtained from the following sources: adriamycin, from Kyowa Hakko Co. Ltd., Tokyo, Japan; vincristine and vinblastine, from Shionogi Co. Ltd., Osaka, Japan.

Cells and cell culture. The human myelogenous leukemia K562 cell line was provided by Dr. Ezaki, Cancer Chemotherapy Center (Tokyo), and its ADM-resistant subline (K562/ADM) was established in our laboratory [18]. Human ovarian cancer cell line A2780 and its ADM-resistant subline (2780AD) was provided by Dr. R. F. Ozols, National Cancer Institute, Bethesda, Md [19]. The acute lymphoblastic leukemia cell line (CCRF-CEM) and its VLB-resistant subline (CEM-VLB100) were provided by Dr. W. Beck, St. Jude Children's Hospital [2]. The human breast cancer cell line, Hattori, was provided by Dr. Shimoyama, National Cancer Center Institute, Tokyo. The subline resistant to ADM (Hattori/ADM) was established in our laboratory. MCF-7, its colchicine-resistant subline (MCF-7/CL10), and a revertant cell line (MCF-7/CL10R) were provided by Dr. Curt, National Cancer Institute, Bethesda, Md [5].

All cell lines were cultured in RPMI 1640 supplemented with 5% fetal bovine serum (Grand Island Biological Co., Grand Island, NY, USA) and kanamycin (100 μg/ml) [18].

Enzyme assay. Cell homogenates were prepared by hypotonic lysis followed by disruption with a Dounce homogenizer. The homogenates were centrifuged at 10,500 *g* for

15 min and the supernatants were used for assay. GST activity was determined as previously described by Habig and Jakoby [7]. All assays were run in triplicate for each homogenate per cell line. One unit of glutathione S-transferase activity is the amount of enzyme catalyzing the conjugation of 1 μmol substrate/min. Protein was determined by the method of Lowry et al. [10], using bovine serum albumin as a standard.

Drug sensitivity. Cells (2×10^4) were cultured at 37° C for 5 h in 2 ml growth medium per tube, after which they were treated with a graded concentration of antitumor agents. The cells were cultured in the presence of the drugs and counted 3 days after drug treatment. The cytotoxic activity of the drug was measured by determining the IC_{50} as previously described [17].

Inhibition of GST by antibody. Inhibition of GST by anti-GST-π antibody was carried out as previously described by Takeoka et al. [16]. Briefly, 1.25–2.5 μl antibody was diluted with 10 m*M* phosphate-buffered 0.9% saline solution to 25 μl, mixed with 50 μl enzyme solution and made up to 250 μl with saline. The mixtures were incubated for 4 h at 4° C, centrifuged at 10,500 *g* for 15 min, and the supernatants were used for assays. Anti-GST-π antibody was generously provided by Drs. Sato and Tsuchida [14], Hirosaki University.

Results

The IC_{50} values of K562 and K562/ADM to antitumor agents are shown in Table 1. K562/ADM was 128-fold more resistant to ADM than the parental cells, K562, and showed marked cross-resistance to VCR, as has been commonly observed for various multidrug-resistant tumor lines. As shown in Fig. 1, there was no increase in GST activity in K562/ADM compared with K562. Similar results were obtained with other human multidrug-resistant cells, 2780AD and CEM-VLB100 (Fig. 2).

In Hattori breast cancer cells, the level of GST was about 12-fold less than the K562, A2780, CCRF-CEM, and their drug-resistant sublines (Fig. 2). The level of GST in the resistant subline, Hattori/ADM, was found to be identical to that in the parental line. Hattori/ADM is 3.3- and 10-fold more resistant to ADM and VCR, respectively (Table 1). Enhancement of GST activity did not occur during the development of drug resistance.

In contrast, a 45-fold increase of glutathione S-transferase activity has been reported in a multidrug-resistant subline of the human breast cancer cell MCF-7 [1]. We examined the GST activity in MCF-7, in a colchicine-resistant subline, MCF-7/CL10, and in a revertant of MCF-7/CL10, MCF-7/CL10R (Fig. 1). Similar to Hattori, the level of GST activity in MCF-7 was lower than that observed in tumor cells used in this study. The level of GST activity in MCF-7 was consistent with that previously reported [1]. There was a 70-fold increase of GST activity in the MCF-7/CL10 (Table 1). Compared with the parental line, MCF-7/CL10 showed a marked cross-resistance to ADM (21-fold) and VCR (26-fold) and thus possessed the typical characteristic phenotype of multidrug-resistant tumor cells. However, compared with K562, CCRF-CEM, A2780, and their resistant sublines, it is clear that the level of GST activity in MCF-7/CL10 was not high enough to explain its drug resistance (Fig. 2). Although the revertant MCF-7/CL10R obtained by passage of MCF-7/CL10 in drug-free medium did not express the phenotype of multidrug resistance, the level of GST was also found to be 70-fold higher than that in MCF-7 (Table 1). It should be noted that the increase of GST activity did not alter the sensitivity of the cell to cytotoxic drugs. These results indicate that GST is not associated with drug resistance in these cell lines, including MCF-7 and its resistant subline.

To elucidate the GST isoenzyme in MCF-7/CL10 and MCF-7/CL10R, activity inhibition was carried out using anti-GST-π antibody. As shown in Fig. 3, antibody directed against GST-π markedly inhibits the enzyme activity in the resistant as well as revertant sublines, indicating that the increase of this enzyme activity was due to the appearance of GST-π.

Discussion

We found that the levels of GST activity in human breast cancer cells Hattori and MCF-7 are significantly lower than those of other tumor cells studied. Although we ana-

Table 1. GST activity and cytotoxicity to ADM and VCR in human tumor cell lines

	GST activity (unit/mg)	IC_{50} (n*M*)[a]	
		ADM	VCR
K562	0.155 ± 0.002[b]	13 ± 1.0	1.6 ± 0.3
K562/ADM	0.136 ± 0.008 (0.88)[c]	1,660 ± 100 (128)	1,030 ± 200 (644)
A2780	0.130 ± 0.011	1.3 ± 0.1	0.3 ± 0.002
2780AD	0.146 ± 0.006 (1.12)	1,300 ± 190 (1,000)	290 ± 12 (970)
CCRF-CEM	0.250 ± 0.038	10 ± 0.29	0.21 ± 0.01
CEM-VLB100	0.208 ± 0.012 (0.83)	97 ± 5.2 (10)	104 ± 1.1 (500)
Hattori	0.011 ± 0.003	17 ± 4.0	0.48 ± 0.18
Hattori/ADM	0.004 ± 0.001 (0.36)	56 ± 2.1 (3.3)	5.1 ± 0.7 (10.6)
MCF-7	0.004 ± 0.001	16.7 ± 3.0	0.93 ± 0.05
MCF-7/CL10	0.279 ± 0.02 (69.8)	401 ± 28 (24)	19.5 ± 2.2 (21)
MCF-7/CL10R	0.283 ± 0.006 (70.5)	6.9 ± 0.9 (0.41)	1.78 ± 0.04 (1.9)

[a] IC_{50} = the concentration of a drug that limits the increase in cell density of a culture to one-half that of control
[b] Mean ± SD of three determinations
[c] Numbers in parentheses represent the degree (*x*-fold) of resistance as compared with that of parental cells

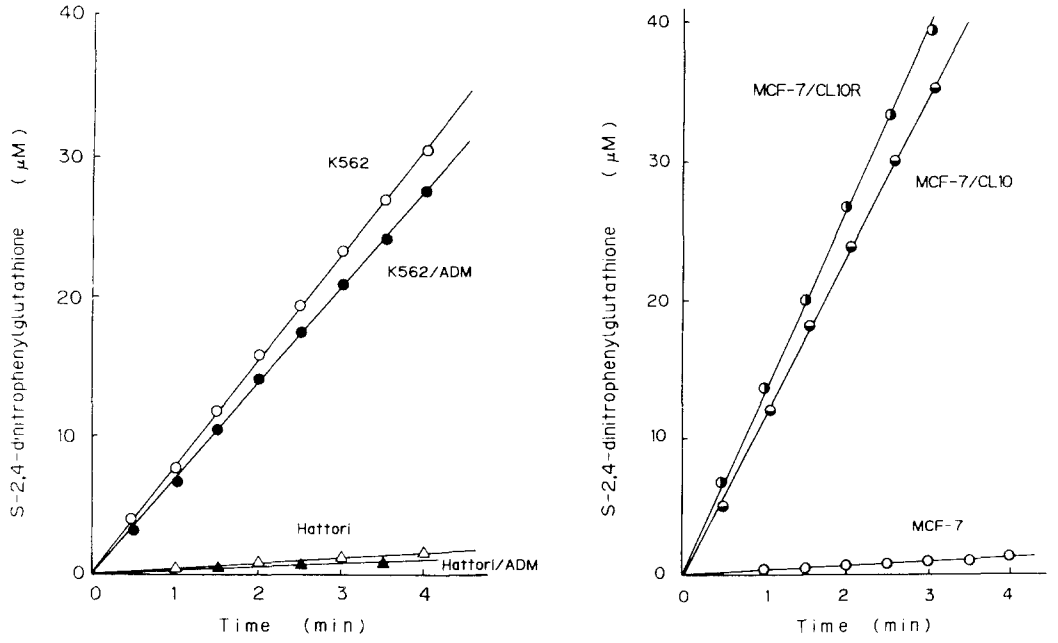

Fig. 1. Reaction of glutathione with 1-chloro-2,4-dinitrobenzene in the presence of GST. Reaction mixtures contained 1-chloro-2,4-dinitrobenzene (1 m*M*), glutathione (1 m*M*), and 50 μg/ml protein in 3 ml 0.1 *M* potassium phosphate buffer (pH 6.5). The reactions were carried out at 25°±0.1° C. A correction for the spontaneous reaction was made by measuring and subtracting the rate in the absence of protein. The concentration of product S-2,4-dinitrophenylglutathione was determined by using an absorption coefficient of 9.6 mM^{-1} at 340 nm [7]

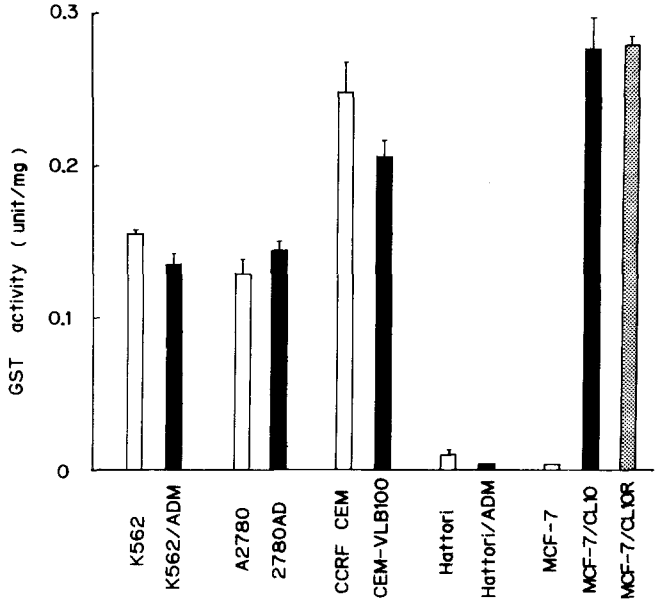

Fig. 2. Comparison of GST activity between drug-resistant and -sensitive cells. The data are the mean of triplicate determinations, and bars represent SD

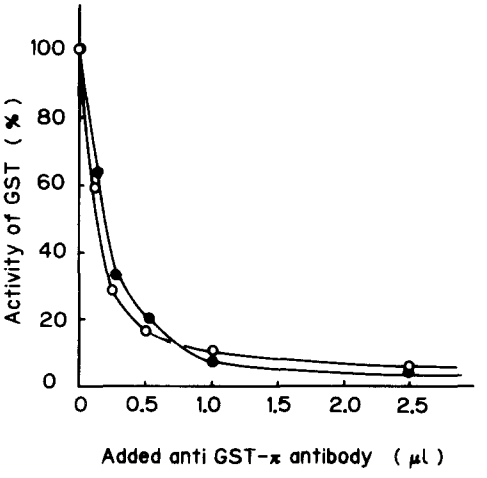

Fig. 3. Activity inhibition of glutathione S-transferase in MCF-7/CL10 and MCF-7/CL10R by anti-GST-π antibody. The activity inhibition was carried out as described in materials and methods. MCF-7/CL10 (○); MCF-7/CL10R (●)

lyzed a limited number of breast cancer lines, the question as to whether or not lower GST activity is common to breast cancer is of great interest. Induction of drug resistance causes divergent cellular responses. A 70-fold increase of GST activity was observed in the resistant subline MCF-7/CL10 but not in the resistant subline Hattori/ADM. In addition, this change was stable in the revertant of MCF-7/CL10, indicating that the induction of GST was irreversible after loss of drug resistance. It has been reported that the induction of GST is due to the transcriptional activation of the gene in the resistant subline of MCF-7 [1]. The level of GST activity in multidrug-resistant MCF-7 is similar to that in other cell lines, and the great increase in GST activity in multidrug-resistant MCF-7 compared with that in the parent MCF-7 could be explained by the extremely low level of GST in MCF-7. In contrast, an increase in GST activity was not seen in the drug-resistant subline of Hattori. Therefore, different cellular responses to cytotoxic stress in human breast cancer cells during the development of drug resistance remain to be determined.

The finding of increased cellular glutathione in a murine cell line resistant to melphalan [15] has suggested that glutathione metabolism could be a factor in the drug-resis-

tant phenotype. Perhaps an increase in GST activity may serve as a protective effect; however, this change was not associated with the development of multidrug resistance. Indeed, the increased level of GST activity in MCF-7/CL10 and MCF-7/CL10R was similar to those observed in other drug-sensitive and -resistant tumor cells, indicating that the level of GST (0.13–0.28 unit/mg) could not influence the cellular response to cytotoxic drugs.

In rat liver, the placental form of GST (GST-π) has been reported as a new marker enzyme for preneoplastic lesions during chemical carcinogenesis [12]. The rat placental form of GST is immunologically related to the human placental form [13]. Therefore, it is of interest that the increase of GST activity in MCF-7/CL10 and MCF-7/CL10R was due to the induction of GST-π during the development of drug resistance.

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