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Glutathione content but not gamma glutamyl cysteine synthetase mRNA expression predicts cisplatin resistance in head and neck cancer cell lines

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Abstract *Purpose*: To correlate cellular glutathione content and γ-glutamyl cysteine synthetase (γGCS) mRNA expression with cisplatin sensitivity in a panel of seven head and neck squamous cancer cell lines. *Methods*: Cisplatin IC_{50} was determined for each cell line using a sodium tetreazolium (XTT) assay. Cellular glutathione content was measured by using a previously reported enzymic method. γGCS mRNA expression was measured using an RNase protection assay. *Results*: Total cellular glutathione was an excellent predictor of cisplatin sensitivity in this series of cell lines. The IC_{50} for cisplatin in the cell line with the highest glutathione concentration was approximately 90 times higher than in the cell line with the lowest glutathione concentration. Regression analysis showed a highly statistically significant positive correlation between cisplatin IC_{50} and cellular glutathione (coefficient of determination $R^2 = 0.81$, $P = 0.0012$). Somewhat surprisingly, in contrast to previous studies in ovarian cancer, γGCS mRNA expression in these cell lines was not significantly predictive of either total cellular glutathione or cisplatin sensitivity ($R^2 = 0.005$, $P = 0.84$). As expected, treatment of resistant cell lines with buthionine sulfoximine resulted in decreased cellular glutathione and enhanced cisplatin sensitivity. *Conclusions*: Our results suggest that glutathione may be an important determinant of cisplatin sensitivity in clinical head and neck cancer. Since cisplatin is the most active chemotherapy drug for the treatment of this disease, this correlation may have important clinical relevance. The lack of correlation between glutathione level and γGCS expression suggests that salvage or alternate synthetic pathways may be critical in these cells.

Key words Head and neck cancer · Glutathione · Cisplatin · γ-Glutamyl cysteine synthetase

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Introduction

In 1995, there were approximately 50 000 new cases of squamous cell cancer of the head and neck diagnosed in the United States, accounting for an estimated 13 000 deaths [1].

Until recently, chemotherapy has been used almost exclusively for palliation in relapsed head and neck cancer, and has had relatively little role in the curative therapy of these tumors. However, roughly 20 000 patients per year will present with recurrent or metastatic disease, which is generally treatable only with chemotherapy, and this is a large and important subset of patients [2]. Partial response rates for methotrexate and most other single agents approximate 30% [3]. In the 1970s, cisplatin was introduced into the chemotherapeutic armamentarium. While the efficacy of cisplatin as a single agent in head and neck cancer was initially found to be only modestly better than other single chemotherapy drugs, it was soon appreciated that chemotherapy combinations based on cisplatin were highly active in head and neck cancers. Overall responses of 70–90% with complete responses of 20–30% have been reported in previously untreated patients when cisplatin is combined with 5-fluorouracil

or other agents such as bleomycin [4–8]. These numbers shift dramatically downward in patients who present for chemotherapy in the setting of relapse. Jacobs, in a well documented study of more than 200 patients treated for relapse at the primary site or the local lymph node field following surgery and radiation, showed only a 32% response rate to the same cisplatin-based chemotherapy [2]. The conclusion to be drawn from these studies is that squamous cell carcinoma of the head and neck in previously untreated patients is extremely chemotherapy-sensitive while the same cancer in patients who have relapsed following surgery and radiation is extremely chemotherapy-resistant.

The majority of patients with head and neck cancer who receive chemotherapy are treated for recurrent or metastatic disease, and since only one-third of them will respond to the treatment, it would be tremendously useful to be able to predict in advance which patients would be likely to benefit from chemotherapy and which not.

A number of mechanisms have been studied regarding the ability of tumor cells to resist the cytotoxic effects of cisplatin [9]. Among these, considerable research has focused on the importance of glutathione (GSH), a nonprotein thiol found ubiquitously in nature [10]. Functionally, GSH and enzymes important in the synthesis and consumption of GSH play many roles particularly in the protection of cells from oxidative insults and in the detoxification of noxious agents including metal compounds such as cisplatin [11, 12]. GSH is synthesized from the amino acids glutamine, cysteine and glycine via two reactions involving the enzymes γ-glutamyl cysteine synthetase (γGCS) and GSH synthetase. γGCS forms γ-glutamyl-L-cysteine from the amino acid precursors L-glutamate and L-cysteine and is inhibited by the amino acid analog buthionine sulfoximine (BSO), causing the depletion of intracellular GSH [13, 14]. γGCS is ATP- and Mg^{2+}-dependent. γGCS is the rate-limiting enzyme in the formtion of GSH and is feedback-inhibited by GSH [15].

Elevated cellular GSH levels have been reported in several tumor types [16]. Increased GSH levels have been reported in tumor cells resistant to a variety of chemotherapy agents [17, 18] as well as in cells with increased resistance to irradiation [19]. Radiation resistance may be a result of the ability of GSH to bind free radicals generated as a result of radiation exposure.

Hosking et al. studied the in vitro sensitivities (by colony forming assays) to various antineoplastic agents of 14 different human tumor parental cell lines and 15 selected drug resistance cell lines. They measured GSH concentrations, and GST activities, and found a positive correlation (statistically significant) between GSH and cisplatin sensitivities in a variety of tumor cell lines including head and neck cancer lines [18]. Similar findings have been reported in ovarian and head and neck cancer by other groups [20, 21].

Godwin et al. have demonstrated in ovarian cancer cell lines that chronic exposure to cisplatin is correlated with emergence of cisplatin resistance over parental cell lines of 30–1000-fold. This is accompanied by 13–50-fold increases in levels of cellular GSH. Marked increases in expression of γGCS mRNA occur in the resistant cell lines. No change in the level of expression of GSH S-transferases are seen. The authors concluded that increases in cisplatin resistance in these cell lines are attributable to increases in the expression of γGCS mRNA [22].

In this study, we examined the correlation between cisplatin sensitivity, and the expression of γGCS and GSH in head and neck cancer cell lines. Additionally, we examined the ability of BSO, a specific inhibitor of γGCS, to reduce cellular GSH and enhance cisplatin sensitivity.

Materials and methods

Cell lines

Seven cell lines were used for this investigation. All were squamous cell carcinomas established in other laboratories from tumors of the aerodigestive tract. Cell lines HN-22A, HN-17B and HN-38 were provided by Dr. Thomas Carey at the University of Michigan Cancer Center, Ann Arbor, Michigan [23]. Lines PCI-13 and PCI-51 were graciously provided by Dr. Theresa Whiteside of the University of Pittsburgh, Pittsburgh, Pennsylvania. Lines SCC-25 and SCC-25CP were initially described by Teicher et al. [24] and were provided by Dr. J. Lazo, University of Pittsburgh. Cell line SCC-25CP was derived from line SCC-25 through repeated exposure to escalating concentrations of cisplatin in vitro.

All cell lines were routinely cultured in IMEM (Biofluids, Rockville, Md.) with 10% fetal calf serum (FCS).

Cisplatin growth assay

Cell lines to be studied were plated in triplicate in 96-well plates at a density of 10 000 cells per well. All cells were plated in IMEM with 10% FCS. The cells were exposed 24 h after plating to aqueous cisplatin at concentrations ranging from 4×10^{-7} to 2×10^{-4} *M*. Experiments were run in parallel in which the cells were exposed to cisplatin continuously or in which the cells were incubated with cisplatin for 1 h, then washed and returned to the basal medium (IMEM with 10% FCS). The cells were grown for an additional 4 days, after which time the cell growth and viability was determined by XTT assay (Boehringer Mannheim, Indianapolis, Ind.) The optical densities of wells which had been treated with cisplatin were then compared with the optical densities of control wells which had not been treated with cisplatin. The IC_{50} was defined as the concentration of cisplatin causing a 50% decrease in control absorbance. The assay was repeated three times. Figure 1 shows the absorbance of treated cells divided by the absorbance of control (untreated) cells. Error bars represent standard error of the mean for each data point. The IC_{50} was calculated graphically from the data shown.

Glutathione assay

Total cellular GSH was determined in all seven head and neck cell lines by the method of Tietze [25]. Briefly, cells were grown to

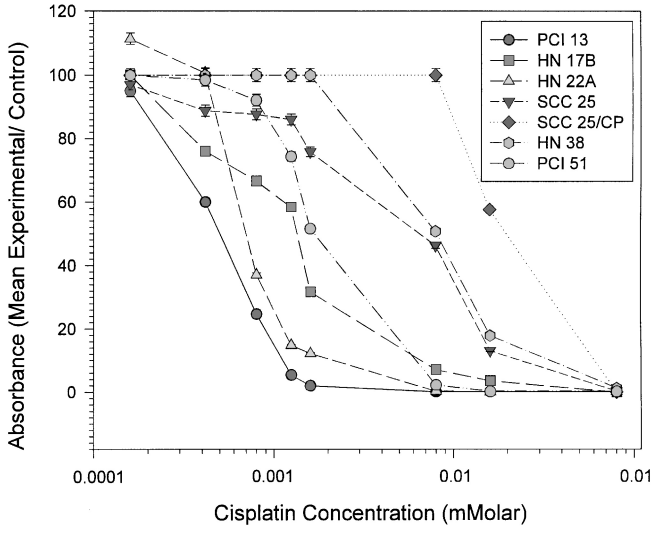

Fig. 1 Cisplatin cytotoxicity assay for the seven head and neck squamous cancer cell lines. Cell lines were plated at equal density on day zero, and treated with increasing concentrations of cisplatin on day 1. Viability for all cell lines was determined by XTT assay on day 6. The cell lines demonstrated a nearly two-log range of cisplatin sensitivity. The IC_{50} for the most resistant cell line, SCC-25CP, was approximately 90 times higher than the IC_{50} for the most sensitive cell line, PCI-13. All cell lines were plated in triplicate. The standard error of the mean is shown for each data point

approximately 80% confluence in a T-75 flask at which time they were scraped from the cell plate into iced phosphate-buffered saline and disrupted by sonication. Total protein in the cell lysates was determined by the method of Bradford with modification (BioRad, Hercules, Calif.). The GSH assay required 15 μg of protein from the cell lysate. All lines were tested in triplicate, and the assays were repeated a total of three times. A standard curve was run with each assay using purified GSH (Sigma, St. Louis, Mo.). The standard error of the mean is shown for each data point.

γGCS RNase protection assay

Cell lines to be analyzed were grown to 80% confluence. Total cellular RNA was extracted by guanidinium/cesium ultracentrifugation [26]. RNA yield was quantified spectrophotometrically and RNA integrity was confirmed by agarose gel electrophoresis.

The γGCS cDNA was provided by Dr. A. Godwin, Fox Chase Cancer Center, Philadelphia, Pa. The cDNA was provided in a bluescript vector, which was linearized with *Ava II*, providing a template for a 220-bp antisense riboprobe. A ^{32}P-labeled riboprobe was synthesized according to the manufacturer's protocol (Promega, Madison, Wis.). Total RNA (20 μg) was hybridized with labeled probe for γGCS and with a riboprobe for γ actin which served as an internal control for sample loading. Samples were hybridized overnight at 50 °C, digested and run on a 6% acrylamide gel. The gel was subsequently dried and exposed to film for 24–48 h.

All assays were repeated twice. γGCS and γ actin mRNA expression was quantitated with a digital phosphorimager (Molecular Dynamics, Sunnyvale, Calif.).

Depletion of GSH with buthionine sulfoximine

To determine whether inhibition of γGCS with the specific inhibitor BSO would result in depletion of GSH and enhance cisplatin sensitivity, cell line HN-38 was selected because of its high level of γGCS mRNA expression and high level of cellular GSH. Cell line SCC-25CP was also selected because it expresses high levels of GSH, but only modest γGCS mRNA. Cellular GSH levels were measured at various time points after treatment with a maximal concentration (10^{-3} *M*) of BSO. (L-Buthionine S-sulfoximine was generously provided by Dr. John Fruehauf, Oncotech, Irvine, Calif.) To test for enhancement of cisplatin sensitivity after BSO treatment, line HN-38 was treated with increasing concentrations of BSO for 48 h to deplete GSH. A fixed concentration of cisplatin was then added to the cells which would not by itself cause significant inhibition of cell growth, based on the data shown in Fig. 1. The BSO-treated cells were allowed to grow in the presence of cisplatin for a total of 7 days. Cell viability was determined by XTT assay. All samples were run in triplicate. The experiment was repeated three times.

Statistical analysis

Cellular GSH concentration (Fig. 2A) and γGCS mRNA content (Fig. 2B) were compared with cisplatin IC_{50} for the seven cell lines analyzed. Regression equations were calculated by Sigma Plot v2.0 (Jandel Scientific, San Rafael, Calif.). Coefficients of determination (R^2) and significance were calculated using Blaise Statistical Software (Blaise Scientific, St. Louis, Mo.).

Results

The panel of head and neck cancer cell lines in this study exhibited a broad range of cisplatin sensitivity. There was an excellent correlation between cisplatin sensitivity and total cellular GSH, but no significant relationship between cisplatin sensitivity and γGCS mRNA expression. BSO treatment resulted in a decline in cellular GSH and enhanced cisplatin sensitivity.

Cisplatin cytotoxicity assay

The seven cell lines demonstrated a nearly two-log range of cisplatin sensitivity. The IC_{50} for the most resistant cell line, SCC-25CP, was approximately 90 times higher than the IC_{50} for the most sensitive cell line, PCI-13. The results of the cytotoxicity assay are shown in Fig. 1.

Cellular GSH/correlation with cisplatin sensitivity

Cellular GSH levels ranged from 40 to 160 nmol/mg protein. The cellular GSH levels for the seven cell lines showed an excellent positive correlation with the IC_{50} for cisplatin (regression equation, $y = 73.4 \log x + 275$; $R^2 = 0.81$, $P = 0.0012$; Fig. 2A). Error bars represent standard error of the mean for cellular GSH content. The experiment was repeated three times. The third experiment is shown. The results of the other two experiments were similar: the coefficients of determination for those assays (R^2) were 0.827 and 0.841, and the *P*-values were 0.0022 and 0.0018, respectively.

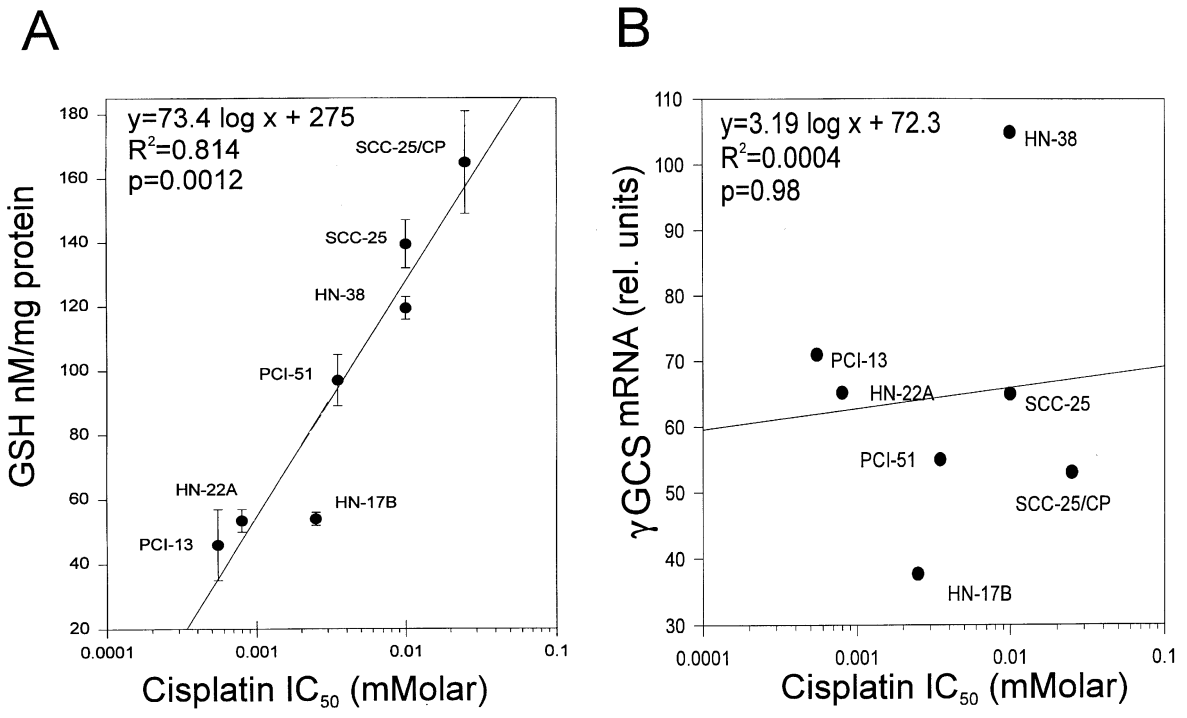

Fig. 2 **A** Total cellular GSH as a function of $IC_{50}$for cisplatin in head and neck tumor cell lines. There was an excellent correlation between cisplatin IC_{50} and total cellular GSH. The calculated regression equation is $y = 73.4 \log x + 275$; $R^2 = 0.81$, $P = 0.0012$. **B** Lack of correlation between γGCS mRNA expression and IC_{50} for cisplatin. Scanning densitometry was used to obtain a numerical value for γGCS mRNA expression after correcting for expression of an internal control mRNA (γ actin). When this value was plotted against IC_{50} for cisplatin, no significant correlation was found (best regression equation $y = 22.0 \log x + 111.6$; $R^2 = 0.005$, $P = 0.84$)

γGCS mRNA expression

Figure 3 shows the results of the RNase protection assay demonstrating the level of γGCS mRNA in the seven head and neck tumor cell lines examined. When levels of γGCS mRNA are corrected for the internal RNA control (γ actin) by photodensitometry, no significant correlation was apparent between γGCS mRNA level and total cellular GSH ($R^2 = 0.005$, $P = 0.84$; Fig. 2B).

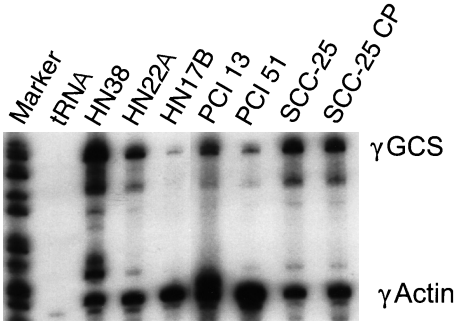

Fig. 3 RNase protection assay for γGCS and γ actin mRNA in head and neck tumor cell lines. Total mRNA (20 μg) was hybridized with both probes as described in Materials and methods. When quantitated by densitometry and corrected for the internal control, γGCS mRNA expression did not correlate significantly with total GSH or cisplatin sensitivity

BSO depletion of GSH

Two cell lines were chosen for this experiment. Both expressed high constitutive levels of GSH, but very different levels of γGCS. We hypothesized that the cell line with the higher level of γGCS mRNA would show a greater decline in GSH following treatment with BSO than the cell line with lower γGCS mRNA expression. The first cell line, HN-38, expressed high constitutive levels of both γGCS mRNA and cellular GSH. Treatment of the cell line with 10 *M* BSO resulted in a 93% decrease in GSH at 24 h. Some rebound was seen in GSH level at 48 h (Fig. 4A). In contrast, cell line SCC-25CP, which expresses high levels of GSH, but modest levels γGCS mRNA, showed a much smaller decrease in GSH content as a result of BSO treatment (50% decrease at 48 h).

As expected, decreased cellular GSH levels as a result of BSO treatment were associated with increased sensitivity to the cytotoxic effects of cisplatin. A sublethal concentration (IC_{20}) of cisplatin for cell line HN-38 showed a modest effect in the absence of BSO, but pretreatment of the cells with increasing concentrations of BSO resulted in marked concentration-dependent inhibition of cell growth with the same concentration of cisplatin (Fig. 4B). BSO by itself had no effect on cell growth.

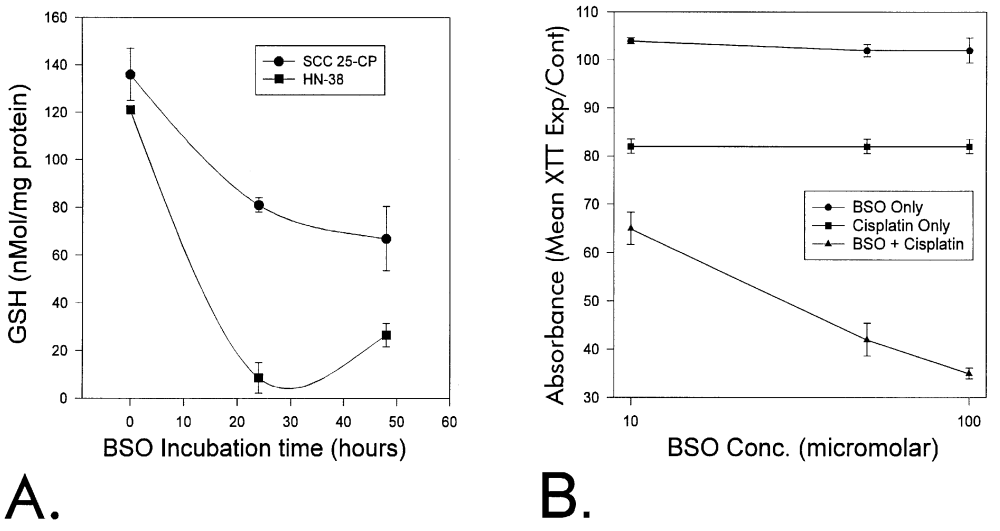

Fig. 4A, B BSO treatment depletes GSH and enhances cisplatin sensitivity. **A** Cell line HN-38 (which expressed high baseline GSH and γGCS) showed a 93% decrease in cellular GSH at 24 h. In contrast, cell line SCC-25CP (which expressed high baseline GSH, but only moderate γGCS) showed only a 50% decrease in GSH at 24 h. **B** Cell line HN-38 was treated with increasing concentrations of BSO alone (●), with an IC_{20} concentration of cisplatin (■) or with cisplatin along with the same concentrations of BSO (▲). BSO alone had no effect on cell growth at any of the concentrations tested. However, cells treated with an IC_{20} concentration of cisplatin were markedly growth inhibited when that sublethal concentration of cisplatin was given in combination with BSO at concentrations greater than 10 μ*M*

Discussion

In this study, we have demonstrated a strong positive correlation between cellular GSH levels and cisplatin resistance in head and neck squamous cell tumor lines in vitro. This supports the hypothesis that cellular GSH is a major determinant of cisplatin resistance in vivo in head and neck cancer. These results agree with previous studies, noteably recent work of Pendyala et al., who showed a strong positive correlation between intracellular GSH content and cisplatin sensitivity in a panel of cell lines derived from other malignancies [27].

In contrast to the results of previous studies by Godwin et al. [22] and Yao et al. [28] in ovarian cancer cell lines, we did not see any consistent direct correlation between γGCS mRNA levels and either total cellular GSH or cisplatin resistance. Some cell lines with high levels of γGCS mRNA expressed expectedly high levels of GSH (HN-38) while others did not. PCI-51 expressed significantly less γGCS mRNA than line PCI-13, but had a higher GSH content and a higher IC_{50} for cisplatin. This suggests that while γGCS is the rate-limiting enzyme in the GSH synthesis pathway [16], it is not the sole determinant of GSH level, at least in head and neck tumor cells. Salvage or alternative synthetic pathways as well as catabolic pathways may significantly influence net GSH content. In a recent study by El-akawi et al., a resistant ovarian carcinoma cell line derived from chronic platinum exposure showed increased GSH, but not γGCS. However, γ-glutamyl transferase (γGT), critical in the GSH salvage pathway, was elevated in cisplatin-resistant cells. Transient exposure to platinum complexes in these cells was also associated with a significant induction of the γGT but not the γGCS message [29].

The idea that pathways not involving γGCS may influence GSH levels can be indirectly tested by treatment with the γGCS inhibitor, BSO. We predicted that a cell line with high expression of γGCS mRNA would show a greater decline in GSH levels with BSO treatment than a cell line with similar GSH levels but low γGCS mRNA expression. The two cell lines with the highest GSH content were treated with 10^{-4} *M* BSO. Line HN-38, which expresses the highest level of γGCS mRNA, showed a marked decline in GSH levels at 24 h (93%). In contrast, line SCC-25CP, which expresses less γGCS mRNA, showed a more modest fall in GSH content (50%) after treatment with the same concentration of BSO.

Additionally, overexpression of γGCS is apparently not the mechanism by which cell line SCC-25CP developed increased resistance to cisplatin as a result of repeated exposure to escalating concentrations of cisplatin. The parental line SCC-25 expressed essentially the same level of γGCS mRNA as the resistant derivative, SCC-25CP.

These considerations are of considerable significance, since BSO has already entered clinical trial [30] and is currently being studied for its ability to reverse resistance to melphalan in ovarian cancer. Since some cell lines are able to increase resistance to cisplatin without increasing γGCS mRNA expression, BSO treatment might be relatively ineffective in some subsets of patients.

Our results do support the idea that GSH may have an important role in chemotherapy resistance in head and neck cancer. Since nearly 20 000 patients with head and neck cancer are treated with chemotherapy in this country each year, this may help guide further studies. GSH has recently been measured by flow cytometry in clinical specimens [31]. Such assays, as well as examination of the expression of other components of the GSH pathway such as GSH S-transferase (s), may yield important predictive information permitting physicians to better tailor cytotoxic therapy for head and neck cancer patients, reducing both morbidity and expense. Additionally, trials of resistance-reversing agents such as BSO could be directed toward patients (whose tumors express elevated γGCS) who might benefit most from them.

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References

1. Wingo PA, Tong T, Bolden S (1995) Cancer statistics 1995. CA Cancer J Clin 45:8
2. Jacobs C, Lyman G, Velez-Garcia E, Sridhar KS, Knight W, Hochster H, Goodnough LT, Mortimer JE, Einhorn LH, Schacter L, Cherng N, Dalton T, Burroughs J, Rozencweig M (1992) A phase III randomized study comparing cisplatin and fluorouracil as single agents and in combination for advanced squamous cell carcinoma of the head and neck. J Clin Oncol 10:257
3. Wheeler RH (1990) Chemotherapy of patients with recurrent head and neck cancers. In: Jacobs C (ed) Carcinomas of the head and neck: evaluation, management. Kluwer Academic, Boston, pp 171–193
4. Kish JA, Weaver A, Jacobs J (1984) Cisplatin and 5-fluorouracil infusion in patients with recurrent and disseminated epidermoid cancer of the head and neck. Cancer 53:1819
5. Hong WK, Shapshay SM, Bhutani R, Craft ML, Ucmalkli A, Yamaguchi K, Vaughan CW, Strong MS (1979) Induction chemotherapy in advanced squamous head and neck carcinoma with high dose cisplatin and bleomycin infusion. Cancer 44:19
6. The Department of Veterans Affairs Laryngeal Cancer Study Group (1991) Induction chemotherapy plus radiation in patients with advanced laryngeal cancer. N Engl J Med 324:1685
7. Jacobs C, Goffinet DR, Goffinet L, Kohler M, Fee WE (1987) Chemotherapy as a substitute for surgery in the treatment of advanced resectable head and neck cancer: a report from the Northern California Oncology Group. Cancer 60(6):1178
8. Hong WK, Lippman SM, Wolf GT (1993) Recent advances in head and neck cancer – larynx preservation and cancer chemoprevention: the Seventeenth Annual Richard and Hinda Rosenthal Foundation Award Lecture. Cancer Res 53(21):5113
9. Andrews PA (1994) Mechanisms of acquired resistance to cisplatin. Cancer Treat Res 73:217
10. Meister A (1988) On the discovery of glutathione. Trends Biochem Sci 13(5):185
11. Mistry P, Harrap KR (1991) Historical aspects of glutathione and cancer chemotherapy. Pharmacol Ther 49:125
12. Meister A (1994) Glutathione, ascorbate, and cellular protection. Cancer Res 54 [Suppl 7]:1969s
13. Hamilton TC, Winker MA, Louie KG, Batist G, Behrens BC, Tsuruo T, Grotzinger KR, McKoy WM, Young RC, Ozols RF, (1985) Augmentation of adriamycin, melphalan, and cisplatin cytotoxicity in drug-resistant and -sensitive human ovarian carcinoma cell lines by buthionine sulfoximine mediated glutathione depletion. Biochem Pharmacol 34(14):2583
14. Meister A (1992) Depletion of glutathione in normal and malignant human cells. J Natl Cancer Inst 84:1601
15. Tew KD (1994) Glutathione-associated enzymes in anticancer drug resistance. Cancer Res 54:4313
16. Ozols RF, O'Dwyer PJ, Hamilton TC, Young RC (1990) The role of glutathione in drug resistance. Cancer Treat Rev 17A:45
17. Russo A, DeGraff W, Friedman N, Mitchell JB (1986) Selective modulation of glutathione levels in human normal versus tumor cells and subsequent differential response to chemotherapy drugs. Cancer Res 46:2845
18. Hosking LK, Whelan RDH, Shellard SA, Bedford P, Hill BT (1990) An evaluation of the role of glutathione and its associated enzymes in the expression of differential sensitivities to antitumor agents shown by a range of tumor cell lines. Biochem Pharmacol 40:1833
19. Moore WR, Anderson ME, Meister A, Murata K, Kimura A (1989) Increased capacity for glutathione synthesis enhances resistance to radiation in *Escherichia coli*: a possible model for mammalian cell protection. Proc Natl Acad Sci USA 86:1461
20. Mistry P, Kelland LR, Abel G, Sidhar S, Harrap KR (1991) The relationships between glutathione, glutathione-S-transferase and cytotoxicity of platinum drugs and melphalan in eight human ovarian carcinoma cell lines. Br J Cancer 64:215
21. Yellin SA, Davidson BJ, Pinto JT, Sacks PG, Qiao C, Schantz SP (1994) Relationship of glutathione and glutathione S-transferase to cisplatin sensitivity in human head and neck squamous carcinoma cell lines. Cancer Lett 85:223
22. Godwin AK, Meister A, O'Dwyer PJ, Huang CS, Hamilton TC, Anderson ME (1992) High resistance to cisplatin in human ovarian cancer cell lines is associated with marked increase of glutathione synthesis. Proc Natl Acad Sci USA 89(7):3070
23. Carey TE (1994) Head and neck tumor cell lines. In: Hay RJ, Park J-G, Gazdar A (eds) Atlas of human tumor cell lines. Academic Press, San Diego, pp 79–120
24. Teicher BA, Holden SA, Kelley MJ, Shea TC, Cucchi CA, Rosowsky A, Henner WD, Frei E (1987) Characterization of a human squamous carcinoma cell line resistant to cis-diammine dichloroplatinum. Cancer Res 47:388
25. Tietze F (1969) Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione. Anal Biochem 27:502
26. Chirgwin JM, Przybla AE, MacDonald RJ, Rutter WJ (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonucleases. Biochemistry 18:5294
27. Pendyala I, Creaven PJ, Perez R, Zdanowicz JR, Raghavan D (1995) Intracellular glutathione and cytotoxicity of platinum complexes. Cancer Chemother Pharmacol 36:271
28. Yao K, Godwin AK, Johnson SW, Ozols RF, O'Dwyer PJ, Hamilton TC (1995) Evidence for altered regulation of γ-glutamyl cysteine synthetase gene expression among cisplatin-sensitive and cisplatin resistant human ovarian cancer cell lines. Cancer Res 55: 4367
29. El-akawi Z, Zandowicz J, Creaven PJ, Abu-hadid M, Perez R, Penyala L (1996) Induction of γ-glutamyl transpeptidase (γGT) mRNA by platinum complexes. Proc Annu Meet Amer Assoc Cancer Res 37:A2755
30. Gallo JM, Brennan J, Hamilton TC, Halbherr T, Laub PB, Ozols RF, O'Dwyer PJ (1995) Time-dependent pharmacodynamic models in cancer chemotherapy: population pharmacodynamic model for glutathione depletion following modulation by buthionine sulfoximine in a phase I clinical trial of melphalan and BSO. Cancer Res 55:4507
31. Chow S, Hedley D (1995) Flow cytometric determination of glutathione in clinical samples. Cytometry 21:68