Cancer Chemother Pharmacol (1995) 37: 168–172

ORIGINAL ARTICLE

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Collateral sensitivity to radiation and CIS-platinum in a multidrug-resistant human leukemia cell line

Received : 7 October 1994/Accepted: 3 February 1995

Abstract Although collateral sensitivity to gamma radiation has previously been described in multidrug-resistant tumor cell lines, we describe here a multidrug-resistant human T-cell acute lymphatic leukemia cell line, L_{100}, which displayed increased sensitivity to both gamma radiation and cis-platinum. *Cis*-platinum cytotoxicity of parental L_0 cells and L_{100} cells was enhanced, whereas radiation sensitivity of L_0 and L_{100} cells was unaltered by glutathione depletion. These results indicate that disparate mechanisms are operative in the collateral sensitivity of L_{100} cells to gamma radiation and cis-platinum.

Key words: Radiation · *Cis*-platinum · Leukemia

Introduction

It has been noted that chemotherapy resistance is often multifactorial and that natural product drug resistance may relate to altered DNA repair, altered glutathione levels and activity of its dependent enzymes and/or altered cytoplasmic drug distribution in addition to overexpression of P-glycoprotein [1, 8,10,15]. Biochemical alterations within a drug-resistant cell population may produce collateral sensitivity to drugs, the mechanisms of action of which relate to the interruption of biochemical pathways which have been perturbed in the generation of primary resistance. Such alterations may also alter the response of cells to radiation [13]. On the other hand, it has recently been observed that certain fractionated X-irradiated cell lines overexpress P-glycoprotein and display the multidrug-resistant (MDR) phenotype [14, 20].

There have been infrequent attempts to take therapeutic advantage of anticipated mechanisms of emerging drug resistance. Wallerstein et al. reported that L1210 leukemia cells in host mice sequentially treated with methotrexate (MTX) and 6-mercaptopurine (6-MP) can be selected to MTX sensitivity by the addition of folic acid to 6-MP and selected to 6-MP sensitivity by the addition of hypoxanthine to MTX. These alterations in sensitivity are related to proportionate alterations in dihydrofolate reductase and hypoxanthine-guanine phosphoribosyl transferase [28]. More recently, it has been shown that doxorubicin/verapamil-selected MDR sublines of human myeloma, RPMI 8226, are collaterally sensitive to nitrosoureas. This sensitivity is due to the loss of gene expression for the DNA repair enzyme, 0^6-methylguanine DNA methyltransferase [9]. Although the understanding of collateral sensitivity in chemotherapeutics is slowly developing, the relationship of the interactions between chemotherapy and radiotherapy responsiveness is poorly understood. We report here an example of collateral gamma-irradiation and *cis*-platinum (CDDP) sensitivity in an MDR human acute leukemia subline.

Materials and methods

Drug sources

Biorad protein determination solution was obtained from Biorad (Richmond, Calif.). DL-Buthionine-(*S*, *R*)-sulfoximine (BSO), reduced glutathione (GSH), hydrogen peroxide, NADPH, 1-chloro-2,4-dinitrobenzene (CDNB), 5,5′-dithio-bis-nitrobenzoic acid (DTNB) and glutathione reductase were purchased from Sigma Chemical Co.(St. Louis, Mo.).

Financially supported by The Jacob Wallerstein Foundation, The Children's Leukemia Research Foundation, The Margaret and Walter Schmid Leukemia Research Endowment and The Marcia Slater Society for Research in Leukemia

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Cell line

Human acute lymphatic leukemia (ALL) GM3639 (Human Genetic Cell Repository, Camden, N.J.) was selected for resistance by continuous exposure to increased concentrations of vincristine (VCR) as previously described. The VCR-resistant subline, designated L_{100} (because of the ability to grow in 100 n*M* VCR), compared to parental cells (designated L_0) overexpresses P-glycoprotein and displays cross-resistance to daunorubicin and to VP-16 [16, 24].

Chemosensitivity

The MTT assay is based on the principle that living cells have the ability to convert a soluble tetrazolium salt (3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, MTT) to an insoluble formazan precipitate [5]. Cells to be tested were harvested and counted by trypan blue exclusion. Suspensions of L_0 and L_{100} cells at concentrations of 9.9×10^3 cells/ml and 6.6×10^4 cells/ml, respectively, were prepared and aliquots of 150 µl (L_0 1500 cells/well, L_{100} 10,000 cells/well) were placed in individual wells in quadruplicate on 96-well microtiter plates (Falcon Plastics). These cell seeding conditions were determined in preliminary experiments which showed differences in doubling time of L_0 cells and L_{100} cells (45.6 ± 8.2 h and 62.5 ± 11.3 h, respectively), and insured log-phase growth and the generation of sufficient absorbance readings in control wells at the time of harvest. After a 2-h incubation period at 37°C in an atmosphere containing 5% CO_2, 10 µl of the drug to be tested was added. The plates were then incubated at 37°C in an atmospheric containing 5% CO_2 for 5 days. After this drug-exposure period, MTT (20 µl, 5 mg/ml) was added to each well. Plates were incubated for 4 h, after which the medium was removed and the resultant formazan crystals solubilized with dimethyl sulfoxide. The optical density (OD) of the solution was then measured at 540 nm using a multiscan spectrophotometer. The surviving fraction was calculated using the formula: mean of test sample/mean of untreated sample (control). Sensitivity to treatment is expressed as the IC_{50}, the concentration required for 50% inactivation.

Radiosensitivity

Radiation response was determined using the MTT assay, which has previously been successfully used for this purpose [6, 12, 22]. All irradiations were performed using a Cs-137 source at the dose rate of 211 cGy/min. For each radiation dose, quadruplicate wells were used. Suspensions of L_0 and L_{100} cells at concentrations of 9.9×10^3 cells/ml and 6.6×10^4 cells/ml, respectively, were prepared and aliquots of 150 µl were placed in individual wells. After a 5-day incubation period at 37°C in an atmosphere containing 5% CO_2, the MTT assay as described above was performed. The data obtained using this assay do not reflect the absolute radiation sensitivity of cells as determined by clonogenic assay [5], but do reflect the relative radiation responses of the cell lines tested as long as the assays are done under identical experimental conditions.

Measurement of glutathione levels

GSH levels were measured using the method of Tietze [26] with modifications, as follows. Cells (5×10^6) were washed once with ice-cold Hanks' balanced salt solution, and an aliquot was taken for determination of protein content. The cell pellet was extracted in 1 *M* perchloric acid for 1 h and subsequently neutralized with KOH. A 100-µl aliquot of the supernatant was added to a semimicrocuvet containing 0.9 ml 0.2 *M* potassium phosphate, 1 m*M* EDTA buffer at pH 7.0, 50 µl of 1.5 mg/ml DTNB, 40 µl of 40 U/ml GSSG reductase and 50 µl of 4 mg/ml NADPH. The reaction rate was measured at 412 nm with a DU-64 Beckman spectrophotometer. GSH concentrations were calculated from a curve generated by known GSH concentrations each time. Protein contents were determined using the method of Bradford [4].

Measurement of glutathione-*S*-transferase activity

Glutathione-*S*-transferase (GST) activity was measured by the thioether formation method of Habig et al. [11], as follows. Cells ($3–5 \times 10^6$) were washed and resuspended in 0.1 *M* potassium phosphate buffer, pH 6.5. The suspension was sonicated for 3 min on ice, then spun in a microcentrifuge for 3 min. A sample of the supernatant was added to a cuvette containing 1 m*M* CDNB and 1 m*M* GSH. The change in OD at 340 nm was measured at 25°C, recorded with a DU-64 Beckman Spectrophotometer and the GST activity was calculated using the expression $E = 9.6/\text{m}M$ per cm. Protein was measured in a separate aliquot using the method of Bradford method [4].

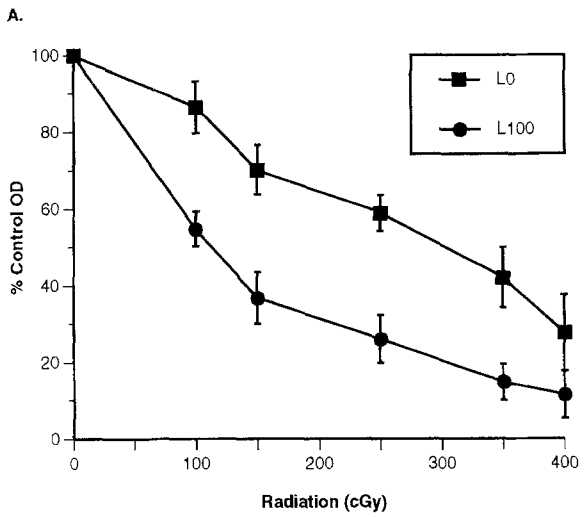
Glutathione depletion studies

Depletion of GSH was achieved in L_0 and L_{100} cells by incubation at a density of 5×10^5 cells/ml in 10 µ*M* BSO for 24 h. Samples were assayed for GSH at 0, 2, 4, 6, 8, 12 and 24 h postincubation. Samples of control untreated cells were also collected at these intervals, since GSH levels are known to fluctuate according to the length of culture. For BSO depletion experiments on CDDP and radiation sensitivity, cells were incubated for 24 h before exposure to CDDP or radiation. All in vitro sensitivity assays were performed three or more times.

Results

Figure 1 compares the cytotoxic effect of gamma irradiation (A) and CDDP (B) between parental drug sensitive L_0 and MDR L_{100} GM3639 cells. The responses of these cells to irradiation and CDDP in terms of IC_{50} values are shown in Table 1 along with data for the responses to VCR, daunorubicin, and VP-16. The results show statistically significant differences between the IC_{50} values for the parental and the MDR subline, which confirm the MDR phenotype of the L_{100} cells, and also show collateral sensitivity of these cells to gamma irradiation and CDDP. The difference between the GSH contents of L_{100} and L_0 cells (6.07 ± 0.05 vs 12.75 ± 1.71 n*M*/mg) approached statistical significance (Table 2). L_{100} cells had significantly greater GST activity than L_0 cells (252.43 ± 16.20 vs 125.23 ± 6.72 n*M*/min per mg; Table 2.)

Figure 2 shows the GSH depletion by BSO in drug-sensitive L_0 cells and resistant L_{100} cells over 24 h. Depletion of GSH by BSO resulted in a significant, approximately 50%, reduction in CDDP cytotoxic IC_{50} values on both L_0 and L_{100} cells but was without effect on gamma radiation sensitivity (Table 3).



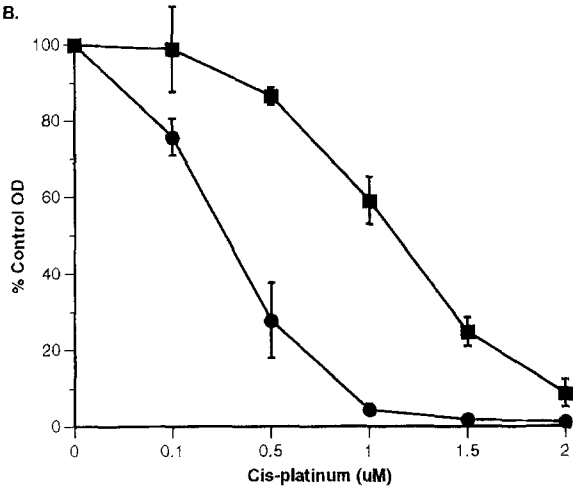


Fig. 1A, B Comparison of (**A**) radiation and (**B**) CDDP sensitivity of L_0 and L_{100} cells

Table 1 Chemotherapy/radiotherapy IC_{50} values

	L_0	L_{100}	*P*-value
VCR (nM)	3.4 ± 0.63	540 ± 10	< 0.001
Daunorubicin (nM)	26 ± 7.8	86 ± 10	< 0.01
VP-16 (nM)	175 ± 19	465 ± 47	< 0.001
CDDP (μM)	1.13 ± 0.08	0.33 ± 0.04	< 0.01
Radiation (cGy)	260 ± 40	120 ± 10	< 0.001

Table 2 GSH and GST levels in L_{100} and L_0 cells

N	GSH (n*M*/mg ± SE)	GST (n*M*/min/mg ± SE)
L_0 3	12.75 ± 1.71	125.23 ± 6.72
L_{100} 4	6.07 ± 0.05	252.43 ± 16.20
	P = 0.056	*P* = 0.001

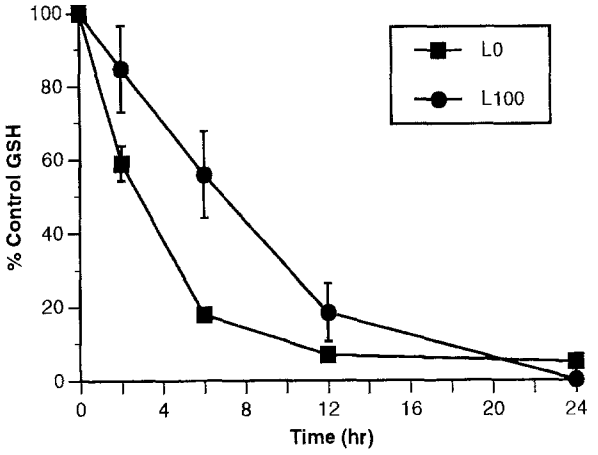


Fig. 2 Comparison of BSO depletion of GSH content L_0 and L_{100} cells

Table 3 Effect of Buthionine sulfoximine on CDDP and radiation IC_{50} values

BSO (μ*M*)	CDDP (μ*M*)		Radiation (cGy)	
	L_0	L_{100}	L_0	L_{100}
0	1.24 ± 0.14	0.40 ± 0.09	260 ± 40	120 ± 10
5	0.82 ± 0.16*	0.26 ± 0.02*	—	—
10	0.69 ± 0.10*	0.22 ± 0.04*	260 ± 40	140 ± 20

*$P < 0.05$ compared with non-BSO-treated

Discussion

In order to develop more effective multimodality therapy for the treatment of malignancies, it is important to understand chemotherapeutic and radiotherapeutic interactions in tumor cells. The relationship between radiation and MDR is unclear [3, 17, 23]. It has recently been noted that in vitro fractionated X-irradiated Chinese hamster ovary cells and in vivo fractionated X-irradiated human lung cancer xenografts overexpress P-glycoprotein and display resistance to natural product chemotherapeutic drugs [14, 20]. It has also been noted that a MDR subline of T-cell human ALL, CEM/VEL_{100}, displays cross-resistance to irradiation. However, a drug-sensitive revertant of CEM/VEL_{100} shows persistence of radiation resistance, indicating that diminished radiation sensitivity, although associated with MDR, is not directly related to it [23]. The development of collateral sensitivity to radiation by transfection of the *MDR*1 gene to 3T3 cells has recently been reported [21].

Although collateral sensitivity of drug-resistant tumor cells to other classes of drugs is well known, there has been limited description of collateral sensitivity between irradiated tumor cells and chemotherapeutic drugs. The best-described interactions in this area seem to relate to CDDP, sensitivity to which can either be

enhanced or diminished by prior irradiation [13]. To our knowledge, there has been only one prior report of an MDR tumor subline with collateral sensitivity to radiotherapy [3], and we now report an example of an MDR tumor subline with collateral sensitivity to both radiotherapy and CDDP. Our results indicate that the mechanisms of these collateral sensitivities appear to be independent.

We originally developed a MDR subline of human T-cell ALL, GM3639, by continuous exposure to initially sublethal and then progressively increasing concentrations of VCR. This ALL subline overexpresses P-glycoprotein and displays the MDR phenotype [16, 24]. Our current data indicate that this leukemia subline has a multifactorial form of resistance relative to its alteration of GSH and GST. MDR L_{100} cells show two- and threefold increased sensitivity to irradiation and CDDP, respectively. The mechanism of this collateral sensitivity is unclear. It has recently been noted that taxol and estramustine enhance radiation sensitivity of human astrocytoma and glioblastoma cells, respectively. This effect relates to cell cycle-specific sensitization rather than MDR since these agents increase the time spent in G_2M of the cell cycle [18, 27, 29]. We have previously noted that the log growth phase distribution of L_0 and L_{100} cells is $G_0 + G_1$ 43% vs 53%, S 39% vs 29% and $G_2 + M$ 18% vs 18%, respectively [25]. L_{100} cells have a slightly reduced S phase, a phase which is associated with radiation resistance. This reduction does not account for the differential in radiation sensitivity that we have observed and other, as yet unknown, mechanisms must be operative.

It has previously been observed that intracellular depletion of GSH and enhanced activity of GST coexist in certain MDR tumor sublines [2, 7, 19]. Cole et al. [7] described an MDR subline, H69AR, of human small-cell lung cancer selected by doxorubicin which failed to overexpress P-glycoprotein but which showed cross-resistance to a variety of natural product drugs. GSH levels of H69AR cells were one-sixth and GST activity tenfold that of parental cells. These cells showed marked collateral sensitivity to BSO, the inhibitor of GSH biosynthesis, which may be related to their diminished GSH content [7]. L_{100} cells had diminished GSH content and significantly elevated activity of GST compared to parental leukemia cells. BSO diminished GSH in a dose-related fashion non-selectively in both L_0 and L_{100} cells. This depletion of GSH correlated with enhanced sensitivity of both parental and MDR cells to CDDP but not to radiation, suggesting that L_{100} cells are collaterally sensitive to CDDP because of their lower GSH content, although we have not excluded possible additional contributing mechanisms such as alterations in DNA repair. Failure of alteration of radiation sensitivity by GSH depletion in aerobic L_0 and L_{100} cells is not surprising in view of their already relatively low GSH content and the presence of oxygen which will over-compete with the thiol for radiation-induced free-radical damage on DNA.

References

1. Batist G, Tulpule A, Sinha BK, Katki AG, Myers CE, Cowan KH (1986) Overexpression of a novel anionic glutathione transferase in multidrug resistant human breast cancer cells. J Biol Chem 261: 15544–15549.
2. Batist G, Schecter R, Woo A, Greene D, Lehnert S (1991) Glutathione depletion in human and in rat multi-drug resistant breast cancer cell lines. Biochem Pharmacol 41(4): 631–635
3. Belli JA, Harris JR (1979) Adriamycin resistance and radiation response. Int J Radiat Oncol Biol Phys 5: 1231–1234
4. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein, utilizing the principle of protein dye binding. Anal Biochem 72: 248–254
5. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB (1987) Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. Cancer Res 47: 936–942
6. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB (1987) Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of radiosensitivity testing. Cancer Res 47: 943–946
7. Cole SPC, Downes HF, Mirski SEL, Clements DJ (1989) Alterations in glutathione and glutathione-related enzymes in a multidrug-resistant small cell lung cancer cell line. Mol Pharmacol 37: 192–197
8. Deffie AM, Alam T, Seneviratne C, Beenken SW, Batra JK, Shea TC, Henner WD, Goldenberg GJ (1988) Multifactorial resistance to adriamycin: relationship of DNA repair, glutathione transferase activity, drug efflux, and P-glycoprotein in cloned cell lines of adriamycin-sensitive and -resistant P388 leukemia. Cancer Res 48: 3595–3602
9. Futscher BW, Campbell K, Dalton WS (1992) Collateral sensitivity to nitrosoureas in multidrug-resistant cells selected with verapamil. Cancer Res 52: 5013–5017
10. Goldenberg GJ, Wang H, Blair GW (1986) Resistance to adriamycin: relationship of cytotoxicity to drug uptake and DNA single- and double-strand breakage in cloned cell lines of adriamycin-sensitive and -resistant P388 leukemia. Cancer Res 46: 2978–2983
11. Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione-S-transferases: the first enzymatic step in mercapturic acid formation. J Biol Chem 249(7130): 7130–7139
12. Hida T, Ueda R, Takahashi T, Watanabe H, Kato T, Suyama M, Sugiura T, Ariyoshi Y, Takahashi T (1989) Chemosensitivity and radiosensitivity of small cell lung cancer cell lines studied by a newly developed 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) hybrid assay. Cancer Res 49: 4785–4790
13. Hill BT (1991) Interactions between antitumour agents and radiation and the expression of resistance. Cancer Treat Rev 18: 149–190
14. Hill BT, Deuchars K, Hosking LK, Ling V, Whelan RDH (1990) Overexpression of P-glycoprotein in mammalian tumor cell lines after fractionated X-irradiation in vitro. J Natl Cancer Inst 82: 607–612
15. Kramer RA, Zakher J (1988) Adriamycin resistance in human breast cancer cells involves both altered transport and oxygen radical detoxification mechanisms. Proc Am Assoc Cancer Res 29: 312
16. Larsson R, Nygren P, Ekberg M, Slater L (1990) Chemotherapeutic drug sensitivity testing of human leukemia cells in vitro using a semiautomated fluorimetric assay. Leukemia 4 (8): 567–571

17. Lehnert SD, Greene D, Batist G (1990) Radiation response of drug-resistant variants of a human breast cancer cell line: the effect of glutathione depletion. Radiat Res 124(2): 208–15
18. Liebmann J, Cook JA, Fisher J, Teague D, Mitchell JB (1994) In vitro studies of taxol as a radiation sensitizer in human tumor cells. J Natl Cancer Inst 86: 441–446
19. Lutzky J, Astor MB, Taub RN, Baker MA, Bhalla K, Gervasoni JE Jr, Rosado M, Stewart V, Krishna S, Hindenburg AA (1989) Role of glutathione and dependent enzymes in anthracycline resistant HL60/AR cells. Cancer Res 49:4120–4125
20. Mattern J, Efferth T, Volm M (1991) Overexpression of P-glycoprotein in human lung carcinoma xenografts after fractionated irradiation in vivo. Radiat Res 127: 335–338
21. Mazzanti R, Fantappie O, Fabrizio P, Pascini S, Ruggiero M (1994) Conferring multiple drug resistance by mdr1 gene transfection increases susceptibility to irradiation and lipid peroxidation. Proc Am Assoc Cancer Res 35: 10
22. Price P, McMillan TJ (1990) Use of the tetrazolium assay in measuring the response of human tumor cells to ionizing radiation. Cancer Res 50: 1392–1396
23. Shimm DS, Olson S, Hill AB (1988) Radiation resistance in a multidrug resistant human T-cell leukemia line. Int J Radiat Oncol Biol Phys 15: 931–936
24. Slater LM, Murray SL, Wetzel MW, Sweet P, Stupecky M (1986) Verapamil potentiation of VP-16–213 in acute lymphatic leukemia and reversal of pleiotropic drug resistance. Cancer Chemother Pharmacol 16: 50–54
25. Slater LM, Sweet P, Hsu TC, Chan PK (1992) Novel nucleolar and nuclear morphology in a vincristine-dependent human leukemia cell line (L_{100}). Exp Cell Res 198: 170–174
26. Tietze F (1969) Enzymic method for qualitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Anal Biochem 27: 502–522
27. Tischler RB, Geard CR, Hall EJ, Schiff PB (1992) Taxol sensitizes human astrocytoma cells to radiation. Cancer Res 52: 3495–3497
28. Wallerstein H, Slater LM, Eng B, Calman N (1972) The return of antimetabolite sensitivity in methotrexate- and 6-mercaptopurine-resistant L1210 murine leukemia by the process of adaptive selection. Cancer Res 32: 2235–2240
29. Yoshida D, Piepmeier J, Weinstein M (1994) Estramustine sensitizes human glioblastoma cells to irradiation. Cancer Res 54: 1415–1417