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ORIGINAL ARTICLE

Paula B. Caffrey · Gerald D. Frenkel

Selenium compounds prevent the induction of drug resistance by cisplatin in human ovarian tumor xenografts in vivo

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Abstract *Purpose*: The development of drug resistance is a major cause for the failure of chemotherapy, particularly in ovarian cancer. Most previous research has focused on approaches to reverse drug resistance once it has arisen, that is, on the use of agents which can make drug-resistant tumors more sensitive to chemotherapy. We have suggested the feasibility of an alternative approach: the use of specific agents to prevent the development of resistance. *Methods*: We designed an in vivo system to assay for the ability of compounds to prevent the induction of resistance by cisplatin. In this system, mice bearing tumors (which originated from A2780 human ovarian tumor cells) were treated with a low dose (2.6 mg/kg) of cisplatin and the tumors rapidly developed resistance to subsequent cisplatin treatment. Cell lines initiated from these tumors retained the resistant phenotype even after several months in culture. *Results*: When either selenite or selenomethionine were administered (i.p., 1.5 mg/kg) close to the time of the initial cisplatin treatment, the induction of resistance was prevented. Similar treatments with sulfite or methionine had no effect on the induction of resistance by cisplatin. Studies in cells from treated tumors have indicated that the selenium compounds may prevent the induction of resistance by preventing a cisplatin-induced increase in glutathione level. *Conclusions*: Selenium compounds specifically prevent the induction by cisplatin of drug resistance in human ovarian tumors in vivo.

Key words Drug resistance · Ovarian tumors · Cisplatin · Selenium compounds

P. B. Caffrey · G. D. Frenkel (✉)
Department of Biological Sciences, Rutgers University, Newark, NJ 07102, USA
e-mail: frenkel@andromeda.rutgers.edu
Tel.: +1-973-3535071; Fax: +1-973-3531007

Introduction

Selenium is an essential dietary trace element which plays an important role in a number of biological processes [24]. There is a long-standing association between selenium compounds and cancer chemoprevention [21]. Many experimental studies in animals have demonstrated the ability of selenium to prevent carcinogenesis, and epidemiological studies have suggested that decreased selenium status in humans is associated with increased risk of cancer [14]. A recent widely publicized chemoprevention study has shown that selenium supplements can decrease the incidence of certain types of cancer [13]. In addition, selenium compounds have been utilized in preventing the nephrotoxic side effects of chemotherapy [4, 5, 20, 29, 30, 31].

We have been investigating the use of selenium compounds in dealing with drug resistance, a major problem in the treatment of human cancer [2]. Chemotherapy is the treatment of choice for many types of tumors; however, after treatment there frequently is a recurrence of the tumor with malignant cells which are resistant to the agent, and often to a variety of drugs [2]. Most previous research has focused on approaches to reverse drug resistance once it has arisen, that is, on the use of agents that can make drug-resistant tumors more sensitive to chemotherapy [33]. We have been investigating an alternative approach to the problem: the use of agents that have the ability to prevent the induction of resistance by the initial drug treatment. Our earlier studies had suggested that selenium compounds are good potential candidates [7] and we have previously described our studies on their ability to prevent melphalan-induced drug resistance in vitro [10, 11] and in a combined in vivo/in vitro system [8, 9].

Cisplatin is a major component of chemotherapy for many types of cancer, including ovarian (National Cancer Institute current recommendations: http://cancernet.nci.nih.gov/clinpdq/soa/Ovarian_epithelial_cancer_Physician.html). However, as is the case with other

drugs, its usefulness has been limited by the development of resistance [22]. We have developed an in vivo system for the rapid induction of cisplatin resistance in human ovarian tumor xenografts. In this report we describe our use of this system to demonstrate that cisplatin-induced drug resistance can be prevented by selenite or selenomethionine but not by their respective sulfur homologues sulfite and methionine.

Materials and methods

Chemicals and cells

Sodium selenite was purchased from BDH; all other chemicals were from Sigma. Human ovarian tumor cells (A2780) were obtained from Dr. Thomas Hamilton, Fox Chase Cancer Center, Philadelphia. Cells were cultured at 37 °C in an atmosphere containing 5% CO_2 in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12, with 10% fetal bovine serum (GIBCO Life Technologies).

Growth of tumors in vivo

All experiments involving animals were approved by the Rutgers University Animal Welfare Committee and were carried out under the supervision of the university veterinarians. Female athymic nude mice were purchased from Harlan Sprague-Dawley (Indianapolis, Ind.) and housed in sterile microisolator cages. At 5–6 weeks of age the animals were inoculated s.c. in the flank with 0.1 ml of a cell suspension containing 5×10^6 A2780 tumor cells. Tumor dimensions were measured with calipers and the volume was calculated using the formula: volume = length × width^2/2. The growth rate of the tumors was calculated by nonlinear regression (exponential growth model) by GraphPad Prism (GraphPad Software, San Diego, Calif.).

Treatments in vivo

When tumor size reached approximately 0.5 ml, animals were inoculated i.p. with 0.1 ml phosphate-buffered saline (PBS) (controls) or 2.6 mg/kg cisplatin in 0.1 ml PBS. After 1 week all animals were inoculated i.p. with 7.2 mg/kg cisplatin. The response of the tumor to the latter treatment was used as a measure of its sensitivity to the drug.

Culture of cells from tumors

Animals were sacrificed 1 week after pretreatment, tumors were removed and fragments were homogenized in 10 ml culture medium containing Penn/Strep and 15% fetal bovine serum. The cell suspension was vortexed thoroughly and diluted to 20 ml, and the cells were seeded into culture flasks. After 24 h, the medium was replenished and incubation was continued (with replenishment of medium every 2 days) for an additional 4–6 days, or until the cells reached approximately 50% confluence. The cells were then trypsinized, and the cultures were maintained in medium containing 10% fetal bovine serum with weekly passage.

Results

In order to examine the ability of selenium compounds to prevent the induction of resistance by cisplatin, we designed an assay system in which treatment of a tumor-bearing animal with cisplatin resulted in the rapid development of resistance to the drug. In this system, nude mice were injected s.c. with ovarian tumor cells and after the tumor reached a size of approximately 0.5 ml received a single i.p. injection of cisplatin (2.6 mg/kg). After 7 days the tumor was tested for sensitivity to cisplatin by examining the effect of a second i.p. dose of cisplatin (7.2 mg/kg). A typical individual result with this protocol is shown in Fig. 1. On day 0, the control animal was pretreated with PBS and the experimental animal was pretreated with 2.6 mg/kg cisplatin. On day 7 both animals were treated with 7.2 mg/kg cisplatin. This dose of cisplatin inhibited the growth of the PBS-pretreated tumor but had no effect on the growth of the cisplatin-pretreated tumor. Thus, the tumor developed resistance as a result of the initial treatment with the drug.

We used this system to test the ability of two selenium compounds, selenite and selenomethionine, to prevent the induction of drug resistance by cisplatin. A total of three treatments with the selenium compound (each 1.5 mg/kg) were administered i.p., the first 24 h before the cisplatin pretreatment, the second 4 h before the cisplatin pretreatment, and the third 24 h after the cisplatin pretreatment. We had previously demonstrated that this dose of selenite is effective in preventing melphalan-induced resistance in these tumors [8]. To examine the specificity of the preventive effect for selenium compounds, we also tested the sulfur homologues of these compounds, using the same doses and schedule. Examples of individual results are shown in Fig. 2A for selenite and sulfite and in Fig. 2B for selenomethionine and methionine. The tumors pretreated with cisplatin

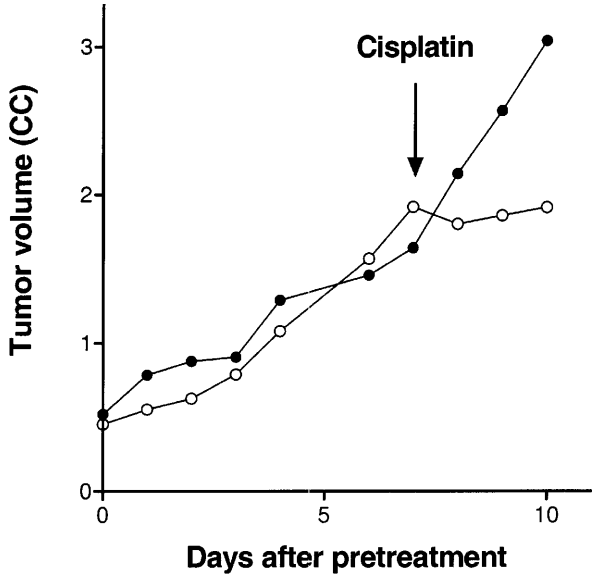

Fig. 1 Induction of drug resistance by cisplatin. Tumor-bearing mice were treated with a single i.p. injection of either PBS (○) or 2.6 mg/kg cisplatin (●) (day 0). Both animals were treated i.p. with 7.2 mg/kg cisplatin on day 7. Tumor volume was calculated as described in Materials and methods

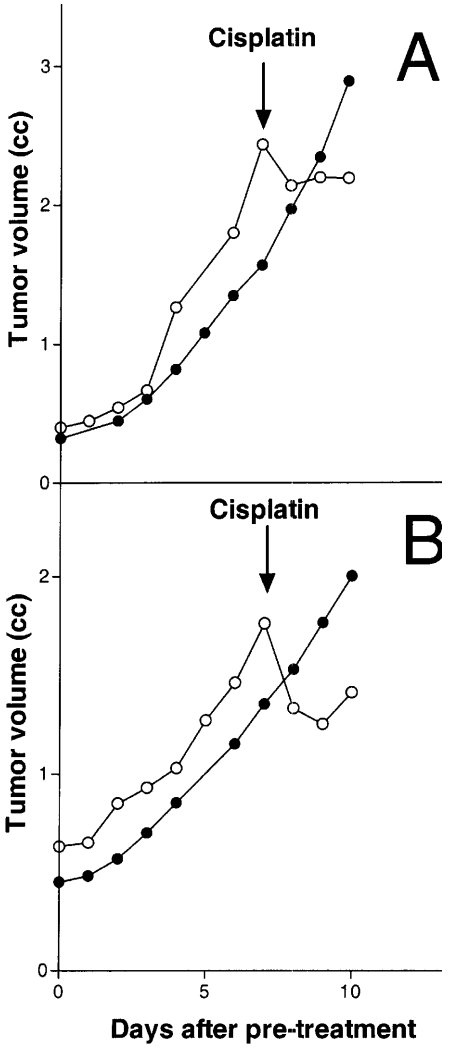

Fig. 2A,B Prevention by selenium compounds of the induction of drug resistance by cisplatin. The experiment was carried out as described for Fig. 1, except that in addition to pretreatment with 2.6 mg/kg cisplatin on day 0, the animal also received three injections of either selenium compound or its sulfur homologue 24 h before, 4 h before, and 24 h after the cisplatin pretreatment (**A** ○ selenite, ● sulfite; **B** ○ selenomethionine, ● methionine)

and either of the selenium compounds remained sensitive to subsequent treatment with cisplatin whereas the tumors pretreated with cisplatin and the homologous sulfur compound became resistant to cisplatin. The complete results of these experiments are shown in Table 1. It is clear that these two selenium compounds consistently and specifically prevented the induction of resistance by cisplatin, but did not themselves affect the sensitivity of the tumors to the drug.

We initiated cell lines from tumors which had been pretreated with either PBS, cisplatin only, cisplatin + selenite or cisplatin + selenomethionine. After approximately 2 months in culture (11 passages), we tested the cells for their sensitivity to cisplatin in vitro. The results (Fig. 3) show that the cells retained the sensitivity/resistance of the tumors from which they originated: cells

Table 1 Selenium compounds prevent the development of cisplatin resistance. Tumor-bearing animals were pretreated on day 0 with either PBS or 2.6 mg/kg cisplatin. Some were also pretreated with 1.5 mg/kg of the indicated selenium or sulfur compound on day –1, day 0 and day 1. All animals were treated with 7.2 mg/kg cisplatin on day 7. Tumor volume was measured for 7 days before and 3 days after the final cisplatin treatment and the growth rates calculated as described in Materials and methods. A tumor was considered resistant if the final treatment with cisplatin produced less than a 40% decrease in growth rate

Pretreatment	Incidence of resistance (resistant tumors/total tumors)	Efficacy of cisplatin[a]
PBS	0/6	82[b]
Cisplatin only	6/6	6[c]
Cisplatin + selenite	0/5	89[b]
Cisplatin + sulfite	3/3	22[c]
Cisplatin + selenomethionine	0/5	113[b]
Cisplatin + methionine	3/3	22[c]
Selenite only	0/4	103[b]
Selenomethionine only	0/4	111[b]

[a] Percent decrease in mean tumor growth rate caused by the final treatment with 7.2 mg/kg cisplatin

[b,c] Values with the same superscript are not significantly different; values with different superscripts are significantly different ($P < 0.02$, t-test)

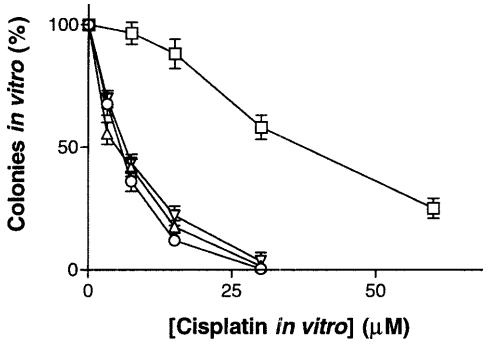

Fig. 3 Effect of treatment of tumors in vivo on the sensitivity of cells to cisplatin in vitro. Cell lines were initiated from treated tumors and maintained in culture as described in Materials and methods. The cells were tested for their sensitivity to cisplatin by measuring the effect of the drug on their ability to form colonies. Cells were seeded into 60-mm dishes (about 200 cells/dish), and allowed to attach for 4 h. The medium was then replaced with serum-free medium containing the indicated concentrations of cisplatin, and incubation continued for 2 h. The medium was then replaced with standard medium with no drug and incubation was continued for 10–14 days, after which the number of colonies on the dish was determined as described previously [6]. The results are expressed as a percentage of the number of colonies in the absence of cisplatin (○ cells from tumors pretreated in vivo with PBS, □ cells from tumors pretreated in vivo with cisplatin only, △ cells from tumors pretreated in vivo with cisplatin and selenite, ▽ cells from tumors pretreated in vivo with cisplatin and selenomethionine)

from cisplatin-treated tumors were resistant in vitro whereas cells from tumors treated with PBS or a combination of cisplatin and selenium compound were not. These results demonstrate that the resistance which developed in vivo is a hereditary property of the tumor cells.

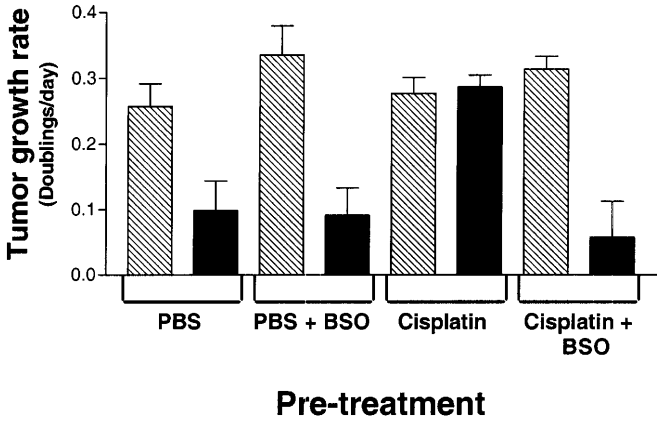

Fig. 4 Effect of BSO on cisplatin-induced drug resistance in vivo. Tumor-bearing mice were pretreated i.p. on day 0 with either PBS or cisplatin (2.6 mg/kg). For those animals that also received BSO treatment, this included i.p injections (27.8 μg/kg) on days 3, 6, 7, and 8 as well as 5.5 ppm in drinking water from day 3 to day 10. On day 7, all animals were injected i.p. with 7.2 mg/kg cisplatin. Tumor growth was measured and growth rates before and after the final cisplatin dose were calculated as described in Materials and methods (▨ growth rate before final cisplatin treatment, ■ growth rate after final cisplatin treatment)

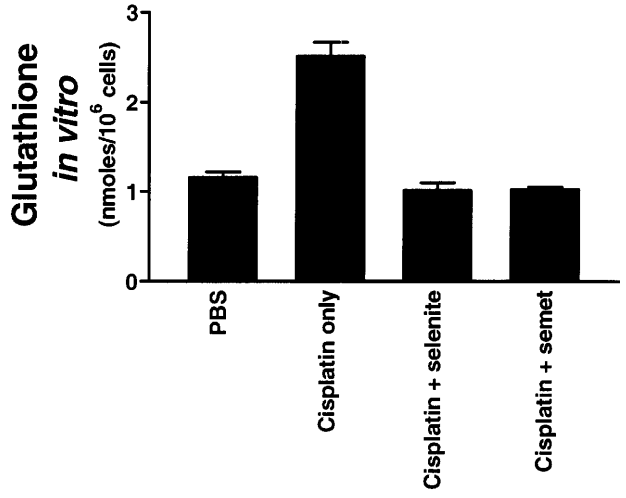

Fig. 5 Effect of treatment of tumors in vivo on the GSH content of cells in vitro. Cell extracts were prepared from the cell lines shown in Fig. 3, and their GSH content was measured by the method of Hissin and Hilf [19]

Glutathione (GSH) has been shown to play a role in cellular resistance to cisplatin [16]. In particular, cisplatin-resistant ovarian tumor cells (including derivatives of the A2780 line) have been found to have elevated levels of GSH, and resistance can be reversed by treatment with buthionine sulfoximine (BSO) [1, 12, 15, 18, 27] which depletes cellular GSH by inhibiting the rate-limiting enzyme in its biosynthesis [17]. In order to determine whether GSH was involved in the cisplatin-induced resistance in our system, we examined the effect of BSO on the sensitivity of cisplatin-pretreated tumors. The results (Fig. 4) show that the cisplatin-induced resistance was reversed by treatment with BSO in vivo. This indicates that increased cellular GSH is (at the least) contributory to the drug-resistant phenotype.

These results suggest a hypothesis for the mechanism by which the selenium compounds prevent the induction of resistance by cisplatin: they prevent a cisplatin-induced increase in GSH levels. However, examining this hypothesis directly in vivo is problematic because of the heterogeneity in the GSH content of tumors [25]. Thus, for this purpose we measured the levels of GSH in the cell lines derived from treated tumors (see above). As shown in Fig. 5, after several months in culture, cells from tumors that had been treated with cisplatin alone had elevated levels of GSH compared to cells from the PBS-treated tumor; in contrast, cells from tumors that had been treated with cisplatin plus selenite or selenomethionine did not have elevated GSH.

Discussion

These results support the hypothesis that selenium compounds prevent the development of resistance in the tumors by preventing the cisplatin-induced increase in the level of GSH. The cellular level of GSH is controlled primarily by its synthesis. Under normal conditions the rate-limiting step in the biosynthetic pathway is the reaction catalyzed by the enzyme γ-glutamylcysteine synthetase [26]. Increased levels of this enzyme are associated with cellular resistance to cisplatin [3, 15, 28]. Thus a reasonable hypothesis is that the selenium compounds prevent a cisplatin-induced increase in the level of this enzyme, perhaps at the level of gene expression. This hypothesis is currently under investigation.

The development of drug resistance is considered to be a major cause of the failure of chemotherapy of ovarian tumors [32] as well as several other cancers, such as breast and lung cancers [2]. Regarding cisplatin, one of the most important drugs in the treatment of ovarian cancer, Kelland [23] has recently stated: "Tumor resistance remains the major factor that limits the clinical effectiveness of cisplatin/carboplatin". There have been many attempts to deal with this problem but "...the key issue of circumvention of tumor resistance to cisplatin has, disappointingly, met with limited clinical success". Thus, a new approach to the problem of drug resistance may be needed. We have suggested that it should be possible to find agents that have the ability to prevent the development of resistance before it arises. Our demonstration of this activity for two selenium compounds provides strong support for this concept.

Selenium compounds have been previously utilized in conjunction with cisplatin in tumor chemotherapy in animal models [4, 5, 29–31] and in a trial in humans [20]. The focus of these studies was the observation that selenium compounds provide a degree of protection from the nephrotoxicity that is associated which cisplatin therapy. (These studies have also shown that there is no chemical interaction between selenite and cisplatin and

administration of selenite does not interfere with the antitumor efficacy of the drug.) In our present experiments, the animals treated with selenium (either alone or in combination with cisplatin), exhibited no overt toxicity other than, in some cases, a temporary weight loss which lasted 3–4 days. Taken together, these findings suggest that selenium compounds may be excellent agents for the prevention of cisplatin-induced drug resistance in a clinical setting.

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References

1. Andrews PA, Murphy MP, Howell SB (1989) Characterization of cisplatin resistance in COLO316 human ovarian carcinoma cells. Eur J Cancer Clin Oncol 25: 619–625
2. Ayash LJ, Herman TS, Frei E (1993) Cancer chemotherapy: clinical evidence for drug resistance. In: Teicher B (ed) Drug resistance and oncology. Marcel Dekker, New York, pp 617–635
3. Bailey HH, Gipp JJ, Ripple M, Wilding G, Mulcahy RT (1992) Increase in γ-glutamylcysteine synthetase activity and steady state messenger RNA levels in melphalan-resistant DU145 human prostate carcinoma cells expressing elevated glutathione levels. Cancer Res 52: 5115–5118
4. Baldew GS, Van-den Hamer CJ, Vermeulen NPE, Goeiji JJM de, Mevie JG (1989) Selenium-induced protection against cis-diaminedichloroplatinum (II) nephrotoxicity in mice and rats. Cancer Res 49: 3020–3023
5. Berry JP, Pauwells C, Tlouzeau S, Lespinats G (1984) Effect of selenium in combination with cis-diamminedichloroplatinum(II) in the treatment of murine fibrosarcoma. Cancer Res 44: 2864–2868
6. Caffrey PB, Frenkel GD (1991) Inhibition of cell colony formation by selenite: involvement of glutathione. Mol Pharmacol 39: 281–284
7. Caffrey PB, Frenkel GD (1994) The development of drug resistance by tumor cells in vitro is accompanied by the development of sensitivity to selenite. Cancer Lett 81: 59–65
8. Caffrey PB, Frenkel GD (1998) Treatment of human ovarian tumor xenografts with selenite prevents the melphalan-induced development of drug resistance. Anticancer Res 18: 3017–3020
9. Caffrey PB, Zhang Y, Frenkel GD (1998) Rapid development of drug resistance in human ovarian tumor xenografts after a single treatment with melphalan in vivo. Anticancer Res 18: 3021–3026
10. Caffrey PB, Zhu M, Frenkel GD (1998) Prevention of the development of melphalan resistance in vitro by selenite. Biol Trace Elem Res 65: 187–196
11. Caffrey PB, Zhu M, Zhang Y, Chinen N, Frenkel GD (1999) Rapid development of glutathione S-transferase-dependent drug resistance in vitro and its prevention by ethacrynic acid. Cancer Lett 136: 47–52
12. Chen G, Hutter K-J, Zeller WJ (1995) Positive correlation between cellular glutathione and acquired cisplatin resistance in human ovarian cancer cells. Cell Biol Toxicol 11: 273–281
13. Clark LC, Combs GF, Turnbull BW, Slate EH, Chalker DK, Chow J, Davis LS, Glover RA, Graham GF, Gross EG, Krongrad A, Lesher JL, Park HK, Sanders BB, Smith CL, Taylor JR (1996) Effects of Se supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. JAMA 276: 1957–1963
14. Combs GF Jr, Combs SB (1986) The role of selenium in nutrition. Academic Press, San Diego, pp 413–461
15. 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: 3070–3074
16. Gosland M, Lum B, Schimmelpfennig J, Baker J, Doukas M (1996) Insights into mechanisms of cisplatin resistance and potential for its clinical reversal. Pharmacotherapy 16: 16–39
17. Griffith OW (1982) Mechanism of action, metabolism and toxicity of buthionine sulfoximine and its higher homologs, potent inhibitors of glutathione synthesis. J Biol Chem 257: 704–713
18. Hamilton TC, Winker MA, Louie KG, Batist G, Behrens BC, Tsuruo T, Grotinger 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: 2583–2586
19. Hissin P, Hilf RA (1976) A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal Biochem 74: 214–226
20. Hu Y, Chen Y, Zhang Y, Zhou M, Song X, Zhang B, Luo L, Xu P, Zhao Y, Cheng G (1997) The protective role of selenium on the toxicity of cisplatin-contained chemotherapy regimen in cancer patients. Biol Trace Elem Res 56: 331–341
21. Ip C (1998) Lessons from basic research in selenium and cancer prevention. J Nutr 128: 1845–1854
22. Ishida H, Kijima H, Ohta Y, Kasani-Sabat M, Scanlon K (1995) Mechanisms of cisplatin resistance and its reversal in human tumors. In: Kellen J (ed) Alternative mechanisms of multidrug resistance in cancer. Birkhauser Press, Boston, pp 225–264
23. Kelland L (1997) Platinum complexes. In: Teicher B (ed) Cancer therapeutics: experimental and clinical agents. Humana, Totowa, pp 93–112
24. Lee BJ, Park SI, Park JM, Chittum HS, Hatfield DL (1996) Molecular biology of selenium and its role in human health. Mol Cells 6: 509–520
25. Lee F, Vessey A Rofstad E, Siemann DW, Sutherland R (1989) Heterogeneity of glutathione content in ovarian cancer. Cancer Res 49: 5244–5248
26. Meister A (1989) Metabolism and function of glutathione. In: Dolphin D, Poulson R, Avramovic O (eds) Coenzymes and cofactors. Glutathione: chemical, biochemical and medical aspects. Wiley, New York, pp 367–374
27. 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: 215–220
28. Mulcahy RT, Bailey HH, Gipp JJ (1994) Up-regulation of γ-glutamylcysteine synthetase activity in melphalan-resistant human multiple myeloma cells expressing elevated glutathione levels. Cancer Chemother Pharmacol 34: 67–71
29. Naganuma A, Satoh M, Yokoyama M, Imura N (1983) Selenium efficiently depressed toxic side effect of cis-diamminedichloroplatinum. Res Commun Chem Pathol Pharmacol 42: 127–134
30. Naganuma A, Satoh M, Imura N (1984) Effect of selenite on renal toxicity and antitumor activity of cis-diamminedichloroplatinum in mice inoculated with Ehrlich ascites tumor cell. J Pharm Dyn 7: 217–220
31. Ohkawa K, Tsukada Y, Dohzono H, Koike K, Terashima Y (1988) Effects of co-administration of selenium and cisplatin (CDDP) on CDDP-induced toxicity and antitumor activity. Br J Cancer 58: 38–41
32. Ozols R, Young R (1984) Chemotherapy of ovarian cancer. Semin Oncol 11: 215–263
33. Tew KD, Houghton PJ, Houghton JA (1993) Preclinical and clinical modulation of anticancer drugs. CRC Press, Boca Raton, pp 1–12