Cancer Chemother Pharmacol (1993) 32: 59–63

Cancer Chemotherapy and Pharmacology

Effect of temozolomide and dacarbazine on O6-alkylguanine-DNA alkyltransferase activity and sensitivity of human tumor cells and xenografts to 1,3-bis(2-chloroethyl)-1-nitrosourea

R. Brian Mitchell*, M. Eileen Dolan

Department of Medicine, Joint Section of Hematology/Oncology, The University of Chicago, Chicago, Illinois, USA

Received 16 July 1992/Accepted 4 December 1992

Abstract. We investigated the ability of 5-(dimethyltriazeno)imidazole-4-carboxamide (DTIC, dacarbazine) and an analogue, temozolomide, to deplete cells or tumors of O6-alkylguanine-DNA alkyltransferase (AGT) and to enhance the antitumor effects of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). Human colon cancer (HT29) cell survival was decreased by almost 1 log when treated with 500 μM temozolomide prior to 150 μM BCNU. Administration of the maximal tolerated dose of DTIC (300 mg/kg) to nude mice carrying HT29 xenografts resulted in complete depletion of AGT activity in tumors at 4 h and 16 h. Administration of 150 mg/kg DTIC caused a 76% reduction in AGT activity at 4 h, but only a 28% reduction at 16 h. The maximally tolerated doses of DTIC and BCNU, alone and in combination, were used to treat nude mice bearing HT29 xenografts. No difference in tumor growth occurred when animals were treated with either BCNU alone (50 mg/kg), DTIC alone (300 mg/kg), DTIC (150 mg/kg) followed by BCNU (12.5 mg/kg), or BCNU (25 mg/kg) followed by DTIC (150 mg/kg). These data suggest that methylating agents such as DTIC may be too toxic to be used in combination with BCNU to deplete tumor alkyltransferase levels effectively and increase the therapeutic index of BCNU.

Introduction

The chloroethylnitrosoureas, including 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), have limited usefulness in human malignant disorders [26]. Evidence suggests that cellular resistance to this class of alkylating agents may be related to the level of O6-alkylguanine-DNA alkyltransferase (AGT) activity. AGT, a DNA repair protein, selectively removes adducts from the O6 position of guanine in DNA by a stoichiometric transfer of the alkyl group to a cysteine moiety within the active site of the protein [29]. Removal of an alkyl group from DNA inactivates the AGT molecule, and restoration of activity requires new protein synthesis [30]. The sensitivity of cell lines to the chloroethylnitrosoureas is correlated with low cellular expression of the AGT protein and to the number of interstrand cross links formed by these agents. There is considerable evidence to suggest that the AGT protein removes or reacts with alkyl groups at the O6 position of guanine prior to formation of lethal interstrand cross-links [2, 12, 17, 20, 32].

Depletion of AGT activity in tumor cells grown in culture results in an increase in the sensitivity of cells to the cytotoxic effects of O6-alkylating agents. Cellular AGT levels can be lowered indirectly by exposure to methylating agents [13, 14, 24, 25, 31] or directly by exposure to O6-methylguanine [7, 15, 19, 34] or O6-benzylguanine [8–10, 27]. The mechanism for depletion by methylating agents involves autoinactivation of the AGT protein following rapid repair of methylated DNA. Exposure of HT29 cells to streptozocin resulted in a depletion of AGT activity, an increase in BCNU-induced interstrand cross-linking, and a 2–3 log enhancement of BCNU cytotoxicity in vitro [13, 31]. Streptozocin has been shown to decrease AGT activity in human lymphocytes [14], indicating the potential for synergy with BCNU in patients. Clinical trials using streptozocin as a biochemical modulator of chloroethylnitrosourea resistance have been prompted by these observations [25, 28].

DTIC has activity in lymphomas and melanoma. Similar to streptozocin, DTIC methylates DNA at 12 sites, including the O6 position of guanine. DTIC has been shown to deplete human lymphocytes of AGT activity [21] and in animal studies was considered one of the most effective depletors of AGT when comparing a series of alkylating agents [24]. Thus, DTIC may be useful as an

This work was supported by National Institutes of Health Grants CA-47228 (M.E.D.) and 5T32-DK-07134 (R.B.M.).

* *Present address:* Department of Medicine, Division of Hematology/Oncology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia, USA

Correspondence to: M. E. Dolan, The University of Chicago, Department of Medicine, Section of Hematology/Oncology, 5841 S. Maryland Ave., MC 2115, Chicago, IL 60637, USA

enhancer of BCNU therapy. DTIC, however, requires metabolic activation to a monomethyl metabolite,5-(3-methyl-1-triazeno)imidazole-4-carboxamide (MTIC) which has been used in in vitro studies [5]. Both MTIC and temozolomide, a methyltriazene that spontaneously decomposes to MTIC, are more cytotoxic towards cells lacking AGT than to cells that possess AGT activity [3, 16, 22].

Although DTIC and BCNU have been used together for many years in the treatment of melanoma, the combination has not been proven superior to therapy with DTIC alone. BCNU is usually administered before or with the first of several daily doses of DTIC [23]. Such schedules are unlikely to take advantage of the potential for a synergistic effect due to AGT depletion by DTIC, since depletion must occur prior to BCNU exposure. Our recent work with O^6-benzylguanine demonstrates the requirement for AGT depletion prior to BCNU exposure [27].

If methylating agents deplete AGT levels in normal tissues to the same degree as in tumor cells, there may be additive toxicity to the host, limiting the usefulness of combining a methylating agent with a chloroethylnitrosourea. Previous studies by Zeller et al. and Skipper have demonstrated synergistic toxicity in animals of combinations of 1-methyl-1-nitrosourea plus BCNU [35] and of DTIC plus 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) [33], but the therapeutic advantage of such combinations has not been fully explored. For this purpose, we have evaluated the effect of temozolomide, a DTIC analogue that does not require metabolic activation, on AGT activity and on the sensitivity of cells to the cytotoxic effects of BCNU. In addition, we have compared the effect of DTIC or BCNU alone with the combination of DTIC prior to BCNU or BCNU prior to DTIC on the growth rate of human colon tumors carried on nude mice.

Materials and methods

Cell lines. The HT29 human colon cancer cell line was a gift from Dr. L. C. Erickson (Loyola University, Maywood, Ill.). Cells were passaged weekly in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, 100 units/ml penicillin, and 100 µg/ml streptomycin, and were maintained in 5% CO_2/95% humidified air. The M14 human melanoma cell line was a gift from Dr. Jon Richards (University of Chicago). Cells were passaged weekly in RPMI 1640 containing 10% fetal calf serum and 100 units/ml penicillin and 100 µg/ml streptomycin.

Drugs and reagents. BCNU (NSC 409962) and temozolomide (NSC 362856) were kindly provided by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. DTIC was purchased from Miles, Inc. (West Haven, Conn.). All other biochemicals were purchased from Sigma Chemical Co. (St. Louis, Mo.). For in vitro use, BCNU was dissolved in ethanol, and then diluted in media, such that the ethanol concentration in cells did not exceed 0.55%. Temozolomide was dissolved directly in culture media immediately prior to use. For animal experiments, BCNU was dissolved in ethanol, then diluted in water immediately before injection to give a final concentration of 3.33 mg/ml in 10% ethanol. DTIC, containing 100 mg citric acid and 50 mg mannitol per 100 mg DTIC, was reconstituted with sterile water. Control animals were treated with the appropriate vehicle.

Cytotoxicity assay. HT29 cells were plated in 80 µl of growth medium in 96-well tissue culture plates (500 cells/well) and allowed to adhere for 24 h. The cells were exposed (in triplicate) to temozolomide for 2 h; then BCNU was added for an additional 2 h, after which the medium was replaced with fresh medium. After 6 days, growth inhibition was measured using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium) assay, as previously described [10, 18]. All results were confirmed by a second, independent experiment.

AGT depletion in cell culture. HT29 cells in log-phase growth (5×10^6 cells per 25 cm^2 flask, in triplicate) were treated with fresh medium containing 200 or 500 µM temozolomide for 4 h. Cell monolayers were then treated with trypsin, washed with PBS, and cell pellets were stored at –80° C until AGT analysis. Average results of two independent experiments are reported.

Alkyltransferase assay. HT29 cells or xenografts were homogenized and sonicated at 4° C. Alkyltransferase activity was measured as removal of ^{3}H-methyl groups from a DNA substrate, which was prepared by allowing ^{3}H-methylnitrosourea to react with calf thymus DNA [8, 11]. Protein was determined by the method of Bradford [1].

Animal treatment. NIH Swiss female nude mice were obtained from Frederick Cancer Research and Development Center (Frederick, Md.) and housed in an isolation facility with water and food provided ad libitum. Animals were injected subcutaneously in each flank with $5-10 \times 10^6$ HT29 cells in 0.1–0.2 ml of Hank's balanced salt solution, and tumors appeared within 2 weeks.

For assay of AGT depletion by DTIC, animals were treated when the tumors reached a size of 400–600 mm^3. After 4 or 16 h, animals were anesthetized with methoxyflurane and sacrificed by cervical dislocation. Immediately after excision, the tumors were frozen in liquid nitrogen and stored at –80° C until AGT analysis. For tumor growth inhibition studies, animals were treated when tumors reached a size of 100–200 mm^3.

Table 1. Toxicity of DTIC and/or BCNU

1st injection (mg/kg)	2nd injection (mg/kg)	Day of maximal weight loss or death	Mean (±SD) maximum weight loss (%)	Surviving fraction[a]
BCNU 25	DTIC 150	4	7± 5	4/4[b]
BCNU 30	DTIC 150	6– 9	17±11	1/4
DTIC 150	BCNU 12.5	3– 7	9± 4	4/4
DTIC 150	BCNU 15	3–10	20±15	3/4
BCNU 50	–	6–11	11± 4	5/5
BCNU 62.5	–	11	30± 6	3/4
DTIC 300	–	4	4± 5	5/5
DTIC 450	–	15–19	21± 8	0/5

[a] Number of surviving animals/number of treated animals
[b] NIH-3 nuce mice

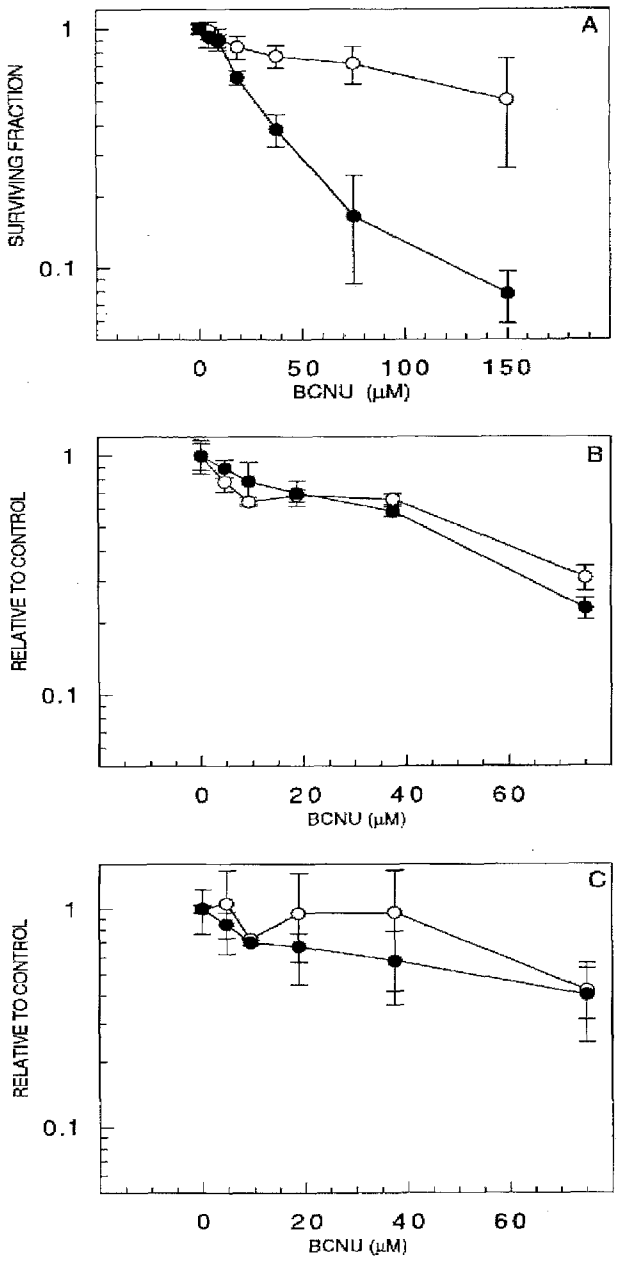

Fig. 1 A – C. HT29 cells (500 per well) were allowed to adhere to 96-well plates for 24 h. After cells were treated with temozolomide for 2 h, BCNU was added at the indicated concentrations for an additional 2 h. Fresh medium was added and cells were incubated for 5 days at 37° C. Growth inhibition was measured by the tetrazolium salt (MTT) assay. **A** Cells were treated without (○) or with (●) 500 μM temozolomide prior to BCNU exposure. **B** Cells were treated without (○) or with (●) 200 μM temozolomide prior to BCNU exposure. **C** Cells were treated without (○) or with (●) 500 μM temozolomide for 4 h after BCNU exposure. Each point represents the mean of three determinations (±SD). Each experiment was repeated with similar results

Animals were weighed and tumors were measured with calipers twice weekly. Tumor volume was calculated using the formula: length × width^2 × 0.53. Statistical analysis was performed using the Kruskal-Wallis analysis of variance method [4].

Toxicity was assessed by survival and maximal weight loss of non-tumor-bearing nude mice. Some toxicity studies as indicated were performed in NIH-3 female nude mice.

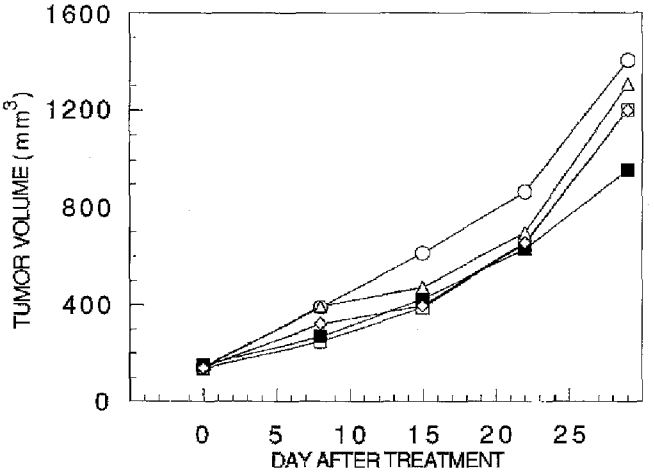

Fig. 2. Nude mice bearing HT29 colon cancer xenografts were treated with the maximally tolerated doses of DTIC, BCNU, or DTIC and BCNU. After tumors reached an average size of 100–200 mm^3, animals were treated with or without 50 mg/kg BCNU (□), 300 mg/kg DTIC (△), 25 mg/kg BCNU followed 1 h later by 150 mg/kg DTIC (◇), 150 mg/kg DTIC followed 1 h later by 12.5 mg/kg BCNU (■), or vehicle alone (○). Tumor size was measured weekly for 4 weeks. Error bars, which are not included, overlap for all groups on each day tumors were measured

Results

Exposure of HT29 cells to 500 μM temozolomide for 4 h, prior to and during exposure to 150 μM BCNU, enhanced the cytotoxicity of BCNU by almost 1 log (Fig. 1 A). No enhancement was seen with 200 μM temozolomide (Fig. 1 B), nor when cells were exposed to 500 μM temozolomide after exposure to BCNU (Fig. 1 C). Exposure of HT29 cells to 200 or 500 μM temozolomide for 4 h resulted in a 31% and 74% reduction in AGT activity, respectively (data not shown). The dose of temozolomide required to achieve a 74% reduction in AGT activity resulted in a 45% inhibition of cell growth (data not shown).

A single i.p. dose of DTIC (300 mg/kg) resulted in nearly complete depletion of AGT activity in HT29 xenografts grown in nude mice at 4 and 16 h. A single dose of 150 mg/kg DTIC depleted AGT levels to 24% of control levels at 4 h after injection, but AGT activity returned to 72% of control levels by 16 h. A single i.p. dose of DTIC (125 mg/kg) depleted AGT levels in M14 tumors to 16% of control levels at 4 and 14 h after treatment.

The toxicity of DTIC, BCNU, or the combinations of DTIC and BCNU in mice is shown in Table 1. Nude mice tolerated a twofold higher dose of BCNU when administered before 150 mg/kg DTIC, compared to administration of BCNU after the same dose of DTIC. Animals were treated with the maximally tolerated doses of either BCNU alone (50 mg/kg), DTIC alone (300 mg/kg), DTIC (150 mg/kg) followed 1 h later by BCNU (12.5 mg/kg), or BCNU (25 mg/kg) followed 1 h later by DTIC (150 mg/kg). As shown in Fig. 2, tumors of animals treated with DTIC prior to BCNU appear to grow at a slower rate than all other groups, but there is no significant difference in tumor size among all groups 28 days after treatment ($P = 0.1$).

Discussion

The current investigation is based on preclinical studies indicating a role for administration of a methylating agent prior to BCNU. We elected to study DTIC because of its activity against lymphoma and melanoma and its demonstrated ability to deplete tissue AGT levels [21, 24]. There is clearly a synergistic effect when cells in culture are exposed to temozolomide prior to BCNU, but the data presented demonstrate that toxic doses of methylating agents (temozolomide or DTIC) are required to achieve significant AGT depletion.

Exposure to 500 μM temozolomide was required to achieve a 74% depletion of AGT activity in HT29 colon tumor cells. D'Incalci et al. [6] found a slight increase in BCNU cytotoxicity when BCNU-resistant L1210 mouse leukemia cells were pretreated in vitro for 1 h with 100 μM temozolomide. In contrast, we found no enhancement of BCNU cytotoxicity when HT29 cells were pretreated for 4 h with 200 μM temozolomide. The requirement for a greater concentration of temozolomide (500 μM) to enhance the cytotoxicity of BCNU in our experiments may be due to the high AGT content of HT29 cells (381 fmol/mg protein). In vivo the maximally tolerated dose of DTIC was required for complete AGT depletion of HT29 xenografts. In order to administer BCNU in combination with DTIC, the dose of DTIC must be decreased, which results in less AGT depletion. However, at tolerable doses, DTIC did not fully deplete AGT in M14 melanoma xenografts either, despite lower AGT levels in M14 tumors.

An alternative to the use of methylating agents to modulate the chemotherapeutic effects of BCNU and other chloroethylnitrosoureas is the use of O^6-alkylguanines. A recently developed analogue, O^6-benzylguanine, has been used to enhance the sensitivity of human tumor cells to the cytotoxic effects of alkylnitrosoureas [8, 10]. We have shown that O^6-benzylguanine effectively depletes AGT levels in human brain tumor xenografts at nontoxic doses and consequently increases the therapeutic index of BCNU [27]. Once comprehensive toxicological evaluation of this agent is completed, clinical trials may be initiated.

AGT activity of host tissues and of the tumor are likely to determine the success or failure of this strategy in any given setting. It is possible that the combination of DTIC followed by BCNU did not inhibit tumor growth more than the maximally tolerated dose of either drug alone because of the high AGT activity of HT29 human tumor cells and the relatively low AGT activity of mouse tissues (e.g., mouse liver: 30 fmol/mg protein) [9]. Rodent tissues are known to have approximately tenfold lower AGT activity than the corresponding human tissue. Thus, the athymic mouse carrying human xenograft tumor may be an unfavorable model to test the ability of AGT modulation to increase the therapeutic index of BCNU. Therapeutic index was not evaluated in M14 tumors because of a low tumor take rate.

In conclusion, the combined administration of the methylating agent DTIC plus BCNU does not increase the therapeutic index of BCNU in nude mice bearing HT29 xenografts. Although the in vitro data suggest synergistic tumor cell kill with these agents, the toxicity to normal tissues is synergistic as well. Respective precautions should be taken when designing clinical trials combining methylating agents with chloroethylnitrosoureas. Furthermore, efforts to utilize non-toxic modulators, such as O^6-benzylguanine, should be encouraged.

Acknowledgements. We would like to thank Ankita Chitre for her technical support.

References

1. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Ann Biochem 72: 248–254
2. Brent TP (1984) Suppression of cross-link formation in chloroethylnitrosourea-treated DNA by an activity in extracts of human leukemic lymphoblasts. Cancer Res 44: 1887–1892
3. Catapano CV, Broggini M, Erba E, Ponti M, Mariani L, Citti L, et al (1987) In vitro and in vivo methazolastone-induced DNA damage and repair in L-1210 leukemia sensitive and resistant to chloroethylnitrosoureas. Cancer Res 47: 4884–4889
4. Daniel WW (1978) Applied non-parametric statistics. Houghton Mifflin, Boston
5. D'Incalci M, Citti L, Taverna P, Catapano CV (1988) Importance of the DNA repair enzyme O^6-alkylguanine alkyltransferase (AT) in cancer chemotherapy. Cancer Treat Rev 15: 279–292
6. D'Incalci M, Taverna P, Erba E, Filippeschi S, Potenza D, Mariani L, et al (1991) O^6-methylguanine and temozolomide can reverse the resistance to chloroethylnitrosoureas of a mouse L1210 leukemia. Anticancer Res 11: 115–122
7. Dolan ME, Young GS, Pegg AE (1986) Effect of O^6-alkylguanine pretreatment on the sensitivity of human colon tumor cells to the cytotoxic effects of chloroethylating agents. Cancer Res 46: 4500–4504
8. Dolan ME, Moschel RC, Pegg AE (1990) Depletion of mammalian O^6-alkylguanine-DNA alkyltransferase activity by O^6-benzylguanine provides a means to evaluate the role of this protein in protection against carcinogenic and therapeutic alkylating agents. Proc Natl Acad Sci USA 87: 5368–5372
9. Dolan ME, Stine L, Mitchell RB, Moschel RC, Pegg AE (1990) Modulation of mammalian O^6-alkylguanine-DNA alkyltransferase in vivo by O^6-benzylguanine and its effect on the sensitivity of a human glioma tumor to 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea. Cancer Commun 2: 371–377
10. Dolan ME, Mitchell RB, Mummert C, Moshel RC, Pegg AE (1991) Effect of O^6-benzylguanine analogues on sensitivity of human tumor cells to the cytotoxic effects of alkylating agents. Cancer Res 51: 3367–3372
11. Domoradzki J, Pegg AE, Dolan ME, Maher VM, McCormick JJ (1984) Correlation between O^6-methylguanine-DNA-methyltransferase activity and resistance of human cells to the cytotoxic and mutagenic effect of N-methyl-N′-nitro-N-nitrosoguanidine. Carcinogenesis 5: 1641–1647
12. Erickson LC, Bradley MO, Ducore JM, Ewig RAG, Kohn KW (1980) DNA cross-linking and cytotoxicity in normal and transformed human cells treated with antitumor nitrosoureas. Proc Natl Acad Sci USA 77: 467–471
13. Futscher BW, Micetich KC, Barnes DM, Fisher RI, Erickson LC (1989) Inhibition of a specific DNA repair system and nitrosourea cytotoxicity in resistant human cancer cells. Cancer Commun 1: 65–73
14. Gerson SL (1989) Modulation of human lymphocyte O^6-alkylguanine-DNA alkyltransferase by streptozotocin in vivo. Cancer Res 49: 3134–3138
15. Gerson SL, Trey JE, Miller K (1988) Potentiation of nitrosourea cytotoxicity in human leukemic cells by inactivation of O^6-alkylguanine-DNA alkyltransferase. Cancer Res 48: 1521–1527

16. Gibson NW, Hartley J, La France RJ, Vaughan K (1986) Differential cytotoxicity and DNA-damaging effects produced in human cells of the Mer$^+$ and Mer$^-$ phenotypes by a series of alkyltriazenylimidazoles. Carcinogenesis 7: 259–265
17. Gonzaga PE, Potter PM, Rafferty JA, Niu T, Margison GM, Ludlum DB, et al (1992) Structure of the guanine-cysteine linkage formed between BCNU-treated DNA and human O^6-methylguanine-DNA methyltransferase. Proc Am Assoc Cancer Res 33: 548
18. Hansen MB, Nielsen SE, Berg K (1989) Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J Immunol Methods 119: 203–210
19. Karran P (1985) Possible depletion of a DNA repair enzyme in human lymphoma cells by subversive repair. Proc Natl Acad Sci 82: 5285–5289
20. Kohn KW (1977) Interstrand cross-linking of DNA by 1,3-bis(2-chloroethyl)-1-nitrosourea and other 1-(2-haloethyl)-1-nitrosoureas. Cancer Res 42: 1450–1454
21. Lee DM, Thatcher N, Margison GP (1991) O^6-alkylguanine-DNA alkyltransferase depletion and regeneration in human peripheral lymphocytes following dacarbazine and fotemustine. Cancer Res 51: 619–623
22. Lunn JM, Harris AL (1988) Cytotoxicity of 5-(3-methyl-1-triazeno)imidazole-4-carboxamide (MTIC) on Mer$^+$, Mer$^+$ Rem$^-$ and Mer$^-$-cell lines: differential potentiation by 3-acetamidobenzamide. Br J Cancer 57: 54–58
23. Mastrangelo MJ, Bellet RE, Kane MJ, Berd D (1992) Chemotherapy of melanoma. In: Perry MC (ed) The chemotherapy source book. Williams and Wilkins, Baltimore
24. Meer L, Schold SC, Kleihues P (1989) Inhibition of the hepatic O^6-alkylguanine-DNA alkyltransferase in vivo by pretreatment with antineoplastic agents. Biochem Pharmacol 38: 929–934
25. Micetich KC, Futscher B, Koch D, Fisher RI, Erickson LC (1992) Phase I study of streptozocin- and carmustine-sequenced administration in patients with advanced cancer. J Natl Cancer Inst 84: 256–260
26. Mitchell EP, Schein PS (1992) Nitrosoureas. In: Perry MC (eds) The chemotherapy source book. Williams and Wilkins, Baltimore
27. Mitchell RB, Moschel RC, Dolan ME (1992) Effect of O^6-benzylguanine on the sensitivity of human tumor xenografts to 1,3-bis(2-chloroethyl)-1-nitrosourea and on DNA interstrand cross-link formation. Cancer Res 52: 1171–1175
28. Panella TJ, Smith DC, Schold SC, Rogers MP, Winer EP, Fine RL, et al (1992) Modulation of O^6-alkylguanine-DNA alkyltransferase-mediated carmustine resistance using streptozotocin: a phase I trial. Cancer Res 52: 2456–2459
29. Pegg AE (1990) Mammalian O^6-alkylguanine-DNA alkyltransferase: regulation and importance in response to alkylating carcinogenesis and therapeutic agents. Cancer Res 50: 6119–6129
30. Pegg AE, Wiest L, Mummert C, Stine L, Moschel RC, Dolan ME (1991) Use of antibodies to human O^6-alkylguanine-DNA alkyltransferase to study the content of this protein in cells treated with O^6-benzylguanine or N-methyl-N′-nitro-N-nitrosoguanidine. Carcinogenesis 12: 1679–1683
31. Pieper RO, Futscher BW, Dong Q, Erickson LC (1991) Effects of Streptozotocin/bis-chloroethylnitrosourea combination therapy on O^6-methylguanine DNA methyltransferase activity and mRNA levels in HT-29 cells in vitro. Cancer Res 51: 1581–1585
32. Robins P, Harris AL, Goldsmith I, Lindahl T (1983) Cross-linking of DNA induced by chloroethylnitrosourea is prevented by O^6-methylguanine-DNA methyltransferase. Nucleic Acids Res 11: 7743–7758
33. Skipper HE (1974) Combination therapy: some concepts and results. Cancer Chemother Rep 4: 137–145
34. Yarosh DB, Hurst Calderone S, Babich MA, Day RS (1986) Inactivation of O^6-methylguanine-DNA methyltransferase and sensitization of human tumor cells to killing by chloroethylnitrosourea by O^6-methylguanine as a free base. Cancer Res 46: 1663–1668
35. Zeller WJ, Berger MR, Weber E (1986) More than additive toxicity of the combination of 1-methyl-1-nitrosourea plus 1,3-bis(2-chloroethyl)-1-nitrosourea in the rat. Cancer Res 46: 1714–1716