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SHORT COMMUNICATION

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Inhibition of O6-alkylguanine-DNA alkyltransferase in animal and human ovarian tumor cell lines by O6-benzylguanine and sensitization to BCNU

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Abstract O6-Alkylguanine-DNA alkyltransferase (O6-AGT) activity in rat ovarian tumor lines O-342 and O-342/DDP was 103.4±18.4 and 240.9±40.2 fmol/mg protein, respectively; thus, cisplatin (DDP) resistance was paralleled by an increase in O6-AGT activity by a factor of approximately 2.3. The DDP-resistant line expressed a collateral resistance to BCNU. Both lines could be sensitized to BCNU by O6-BG, with sensitization factors of 6.0 and 2.1, respectively. In neither line did depletion of O6-AGT have any sensitizing effect towards DDP. In the human ovarian cancer lines SK-OV-3 and OAW 42, O6-AGT activity was 337.6±18.2 and 180.0±39.9 fmol/mg protein, respectively; in these lines depletion of O6-AGT activity by O6-BG treatment resulted in sensitization factors of 3.0 and 4.1, respectively. The increase in sensitivity of ovarian tumor cell lines against a chloroethylating agent by O6-AGT depletion and possible pharmacological advantages of regional (i.p.) administration of this combination might be beneficial in advanced ovarian cancer.

Key words Ovarian cancer
O6-alkylguanine-DNA alkyltransferase
Carmustine resistance

Abbreviations BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea) · CENU 2-chloroethylnitrosourea · DDP cisplatin ED_{50}, the effective dose required to inhibit colony formation or cell proliferation by 50% · O6-AGT O6-alkylguanine-DNA alkyltransferase · O6-BG O6-benzylguanine SF sensitization factor

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Introduction

In the treatment of advanced ovarian cancer, cisplatin (DDP)-containing regimens have an outstanding role; alkylating agents (e.g., cyclophosphamide, melphalan, treosulfan) are also used in the treatment of this disease and are part of various combination-treatment schedules. 2-Chloroethylnitrosoureas (CENUs), on the other hand, have no part in the treatment of ovarian cancer. Clinical trials with BCNU [1,3-bis(2-chloroethyl)-1-nitrosourea] revealed poor response or lack of response to this drug [8, 11]. A phase II study with CCNU revealed one complete remission and one partial remission [duration of response (weeks) 12+; 53+] among five evaluable cases [10], indicating that at least isolated cases of ovarian cancer are sensitive to this class of compounds. Since sensitivity of animal and human tumors to CENUs appears to be related to the activity of the repair enzyme O6-alkylguanine-DNA alkyltranferase (O6-AGT), which removes alkyl groups from the O6-position of guanine before interstrand crosslinks are formed [15], we determined the activity of this enzyme in two rat ovarian tumor cell lines (O-342, O-342/DDP) and in two human ovarian cancer cell lines (SK-OV-3, OAW 42). Since O6-BG appears to be the most active inhibitor of O6-AGT and increases the cytotoxicity of CENUs in vitro and in vivo [4–6], the effect of O6-AGT depletion by O6-BG on the sensitivity of these cell lines to BCNU was elucidated.

Materials and methods

Drugs and chemicals

O6-Benzylguanine (O6-BG) was kindly provided by R. C. Moschel (NCI, Frederick Cancer Research and Development Center, Frederick, Md., USA). It was dissolved in DMSO at a concentration of 100 mM and stored at −20 °C. BCNU was kindly provided by G. Eisenbrand and co-workers (University of Kaiserslautern, Germany); it was dissolved in ethanol immediately before use.

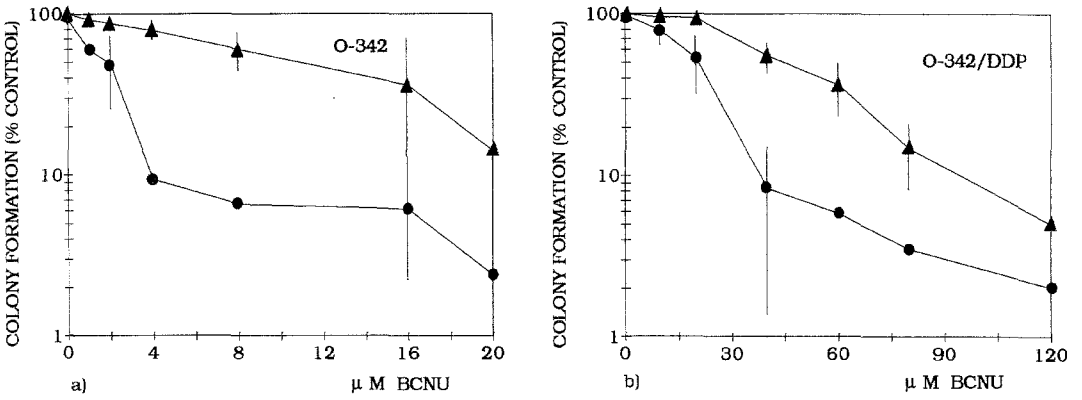


Fig. 1a, b Enhancement of BCNU-cytotoxicity by O6-benzylguanine (O6-BG) (▲ BCNU; ● BCNU plus O6-BG) in animal ovarian tumor cell lines (each *point* represents the mean of three determinations, ±SD)

Tumor cell lines

Ovarian tumor O-342 (host: rat) was originally induced in a pregnant BD IX rat by i.p. injection of ethylnitrosourea and was classified as a granulosa cell tumor that following transplantation to syngeneic recipients was highly malignant with infiltrating growth. Resistance of O-342 to cisplatin was induced by stepwise increasing DDP concentrations; the resistant line was designated as O-342/DDP and was treated continuously with 8 μM DDP (Pharmachemie; Haarlem, The Netherlands). Passage of both lines was performed weekly [RPMI 1640 (Gibco, Eggenstein, Germany), 100 IU/ml Penicillin (Gibco), 100 μg/ml streptomycin (Gibco) + 10% FCS (Boehringer Mannheim, Germany)].

SK-OV-3 was isolated from the ascitic fluid of a 64-year-old patient (treatment: Thiotepa) [9]; it was maintained in Ham's F12 with 20% FCS.

OAW 42 was derived from the ascitic fluid of a 46-year-old patient with serous cystadenocarcinoma of the ovary who had a relapse after treatment with cisplatin and abdominal radiation [18]; this line was maintained in MEM (Gibco) supplemented with 10% FCS. Both human lines were kindly provided by H. Löhrke (German Cancer Research Center, Division 0418).

Antitumor activity

For the determination of cytotoxicity a colony forming assay (O-342, O-342/DDP, OAW 42) or a proliferation assay (SK-OV-3) were used. Cells were seeded into 6-cm Falcon plates (Becton-Dickinson, Plymouth, UK) at a density of 1×10^3 (O-342), 2×10^3 (O-342/DDP, SK-OV-3) or 3×10^3 (OAW 42) cells/plate 1 day before treatment. For each concentration, three plates were used; experiments were usually performed in triplicate. One hour before exposure to BCNU or DDP, O6-BG was added to give a final concentration of 20 μM. After exposure to BCNU or DDP for 2 h, the medium was changed and O6-BG (same concentration) was added for a further 16 h. Cells were allowed to grow for an additional 5–8 days at 37 °C in 5% CO_2 and 99% relative humidity; then plates were washed in PBS (Gibco), dried for 1 day and stained using May-Grünwald (3 min; Marck, Darmstadt, Germany) and Giemsa (20 min; Merck) solutions. Colonies (>50 μm) or cells (SK-OV-3) were counted using an image analyzer (AMS 40-10, Analytical Measuring Systems, Cambridge, UK).

Plating efficiency (mean ± SD) of O-342, O-342 DDP, and OAW 42 were 79±11%, 71±8%, and 23±4%, respectively.

The protein concentration was determined using the method of Bradford [1]. Determination of O6-AGT activity was performed according to Myrnes et al. [13]; briefly, activity of O6-AGT was assessed by counting radioactivity of ^{3}H-labeled methyl adducts transferred from methylated DNA to the repair protein.

Results

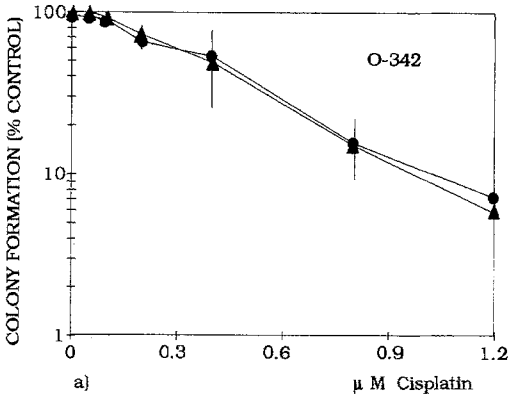
Activity of O6-AGT in O-342 cells was 103.4±18.4 (mean ± SD) fmol/mg protein; activity of O6-AGT in the DDP-resistant line O-342 DDP was increased by a factor of approximately 2.3 (240.9±40.2). Treatment with O6-BG (20 μM, 1 h) reduced O6-AGT activity in O-342 and O-342/DDP cells to 13.0±10.0 and 12.4±0.9 fmol/mg protein, respectively (Table 1).

Exposure of O-342 cells to BCNU (2 h) alone resulted in an ED_{50} of 11.3 μM (Fig. 1a); treatment of O-342/DDP cells with BCNU alone yielded an ED_{50} value of 45.3 μM (Fig. 1b). Combination treatment of O-342 and O-342/DDP with O6-BG (20 μM, 19 h) plus BCNU (concentration range: 0–20 μM and 0–120 μM, respectively) resulted in BCNU ED_{50} values of 1.9 μM and 21.6 μM, respectively (Fig. 1). Thus in O-342 and O-342/DDP the sensitization factor (SF; = ED_{50} of BCNU alone divided by ED_{50} of BCNU combined with O6-BG) was 6.0 and 2.1, respectively (Table 1). After pretreatment of O-342 with O6-BG,

Table 1 Inhibition of O6-AGT activity and sensitization to BCNU

Cell line	O-342	O-342/DDP	SK-OV-3	OAW 42
Activity of O6-AGT (fmol/mg protein)	103.4±18.4	240.9±40.2	337.6±18.2	180.0±39.9
Activity of O6-AGT after exposure to O6-BG (fmol/mg protein)	13.0±10.0	12.4±0.9	21.1±15.3	13.0±7.7
ED_{50} of BCNU (μM)	11.3	45.3	22.2	33.0
ED_{50} of BCNU after O6-AGT inhibition	1.9	21.6	7.4	8.0
Sensitisation factor[a]	6.0	2.1	3.0	4.1

[a] ED_{50} (BCNU) without O6-BG ÷ ED_{50} (BCNU) with O6-BG



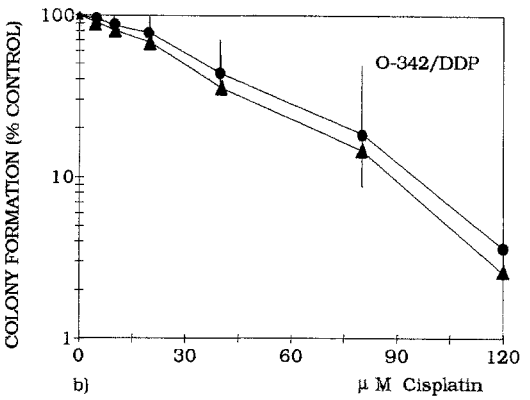


Fig. 2a, b No enhancement of cisplatin cytotoxicity by O^6-BG pretreatment in O-342 or O-342/DDP (▲ cisplatin; ● O^6-BG plus cisplatin)

the dose-response curve of BCNU showed plateau formation at the ED_{10} level between 4 and 16 μM, suggesting the presence of a resistant fraction in this cell line.

Sensitivity of both lines towards DDP is depicted in Fig. 2a and b. The ED_{50} of DDP in O-342 was 0.39 μM, and that in O-342/DDP, 31.1 μM, revealing the high sensitivity of O-342 and the pronounced resistance of O-342/DDP to cisplatin (resistance factor approximately 80). In contrast to combination treatment of both lines with O^6-BG plus BCNU vs BCNU alone (Fig. 1), combination treatment with O^6-BG plus DDP had no sensitizing effect on either line compared with DDP alone (Fig. 2).

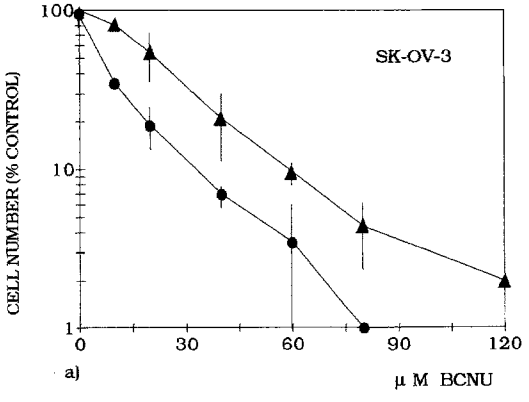
The levels of O^6-AGT activity in SK-OV-3 and OAW 42 cells are also given in Table 1. O^6-AGT activity was 337.6±18.2 fmol/mg protein in untreated SK-OV-3 cells and 180.0±39.9 fmol/mg protein in untreated OAW 42 cells; after exposure to O^6-BG (20 μM, 1 h), O^6-AGT activity was reduced to 21.1±15.3 and 13.0±7.7 fmol/mg protein, respectively. Sensitization of both human ovarian cancer lines against BCNU by treatment with O^6-BG is shown in Fig. 3. Exposure of SK-OV-3 cells to BCNU without and with O^6-BG (20 μM, 19 h) resulted in ED_{50} values of 22.2 and 7.4 μM BCNU, respectively (SF = 3.0; Fig. 3a). Exposure of OAW 42 to the same treatment resulted in ED_{50} values of 33 and 8 μM BCNU, respectively (SF = 4.1).

Discussion

All four lines investigated had significant activity of O^6-AGT. After exposure to O^6-BG, in all lines O^6-AGT activity was reduced to values near baseline activity. In all four lines sensitization to BCNU was observed following additional exposure to O^6-BG. In O-342, O-342/DDP, SK-OV-3 and OAW 42 sensitization factors of 6.0, 2.1, 3.0 and 4.1, respectively, were observed.

Depletion of O^6-AGT by O^6-BG in O-342 had no sensitizing effect towards DDP. This is in line with data published by Dolan et al. [5], who showed that the human colon tumor cell line HT29 could not be sensitized to DDP by pretreatment with O^6-BG. The same was the case in the resistant line O-342/DDP, although in this line development of DDP resistance was paralleled by a significant increase in O^6-AGT activity compared with that in O-342 (240.9 vs 103.4 fmol/mg protein). On the other hand, this increase in O^6-AGT-activity in O-342/DDP cells appears to be responsible for the development of cross-resistance to BCNU in this line (ED_{50} of BCNU = 45.3 μM vs 11.3 μM in O-342).

Fig. 3a, b Enhancement of BCNU cytotoxicity by O^6-BG in human ovarian cancer cell lines (▲, BCNU; ●, BCNU plus O^6-BG)



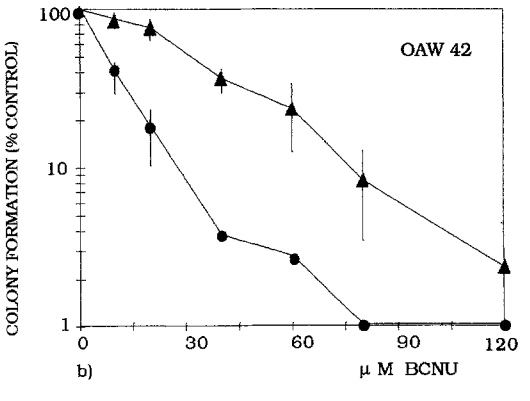


Table. 1 shows that the ratio of BCNU ED_{50} values in O-342/DDP and O-342 was approximately 4 without O^6-AGT depletion, increasing to approximately 11.4 after O^6-AGT depletion. Thus, after O^6-AGT depletion, O-342/DDP cells are still less sensitive to BCNU than the parental line O-342 without O^6-AGT depletion (ED_{50} = 21.6 μM vs 11.3 μM). This implies that response of O-342 to BCNU is mainly determined by O^6-AGT. In O-342/DDP, on the other hand, additionally other mechanisms of resistance to BCNU than O^6-AGT activity were induced by preceding induction and maintenance of resistance to DDP. This has in fact been demonstrated in earlier investigations, in which higher GSH and GSSG levels and higher GSH reductase activity were observed in O-342/DDP than in O-342 [2, 21]. The presence of a resistant fraction in line O-342 might be due to different subpopulations. In O-342 cells a hyperploid karyotype predominates, while in O-342/DDP cells a near-diploid karyotype was observed [3]. It might be speculated that a diploid subline of O-342 is not only DDP-resistant [3] but also BCNU-resistant. This suggestion would be supported by observations of other authors in a heterogenous population of glioma cells: near-diploid cells appeared to be most capable of surviving sublethal doses of a CENU, while hyperploid (3n and 4n) clones disappeared following drug exposure [16].

Recently, Tagliabue et al. [17] reported that the O^6-AGT level in two human ovarian cancer xenografts was a relevant parameter for CENU response; both tumors showed a high O^6-AGT level and an absence of sensitivity towards BCNU [17]. The present investigation in two human ovarian tumor cell lines with pronounced O^6-AGT activity demonstrates that depletion of O^6-AGT leads to sensitization to a CENU underlining that O^6-AGT is an important factor for BCNU resistance in human ovarian tumor cells.

The attempt to sensitize tumors to CENUs by pretreatment with a methylating agent is compromised by the pronounced combination toxicity in normal tissues [19, 20], and it has led in clinical studies to the reduction of the maximal tolerated dose (MTD) of the CENU [12, 14] and the appearance of lethal complications [7, 12]. Since i.p.-administered drugs are able to penetrate into intraperitoneally located tumor tissue, regional application might increase the therapeutic index of this approach above that attainable with systemic administration.

Altogether, the increase in sensitivity of ovarian tumor cell lines against a CENU by O^6-AGT depletion and certain pharmacological advantages of regional (i.p.) administration of drugs suggest that i.p. treatment with O^6-BG and a CENU might be beneficial in the treatment of advanced ovarian cancer.

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