[CANCER RESEARCH 53, 5663–5668, December 1, 1993]

Topoisomerase II Inhibition and Altered Kinetics of Formation and Repair of Nitrosourea and Cisplatin-induced DNA Interstrand Cross-Links and Cytotoxicity in Human Glioblastoma Cells[1]

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ABSTRACT

By altering the accessibility of DNA sequences for alkylation or platination, and/or for subsequent repair, topoisomerase II can potentially affect the level of DNA interstrand cross-links induced in cells by bifunctional agents. In this study, we investigated the extent to which inhibition of topoisomerase II activity in a human glioblastoma multiforme cell line alters the kinetics of both the formation and the repair of total genomic DNA interstrand cross-links, as well as the sensitivity of the tumor cells to *cis*-diamminedichloroplatinum II (*cis*-DDP) and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). Cells were incubated with and without 200 μM novobiocin, a known topoisomerase II inhibitor, for 24 h, followed by exposure to 50 μM BCNU and 25 μM *cis*-DDP. DNA interstrand cross-linking was determined at various time points over 72 h, using a modified ethidium bromide-DNA binding assay. Sensitivity of the cells to *cis*-DDP and BCNU was also determined with and without novobiocin pretreatment with 200 μM novobiocin. This concentration of novobiocin showed no significant direct cytoxicity, although it inhibited topoisomerase II activity in tumor cell nuclear extracts by 73%. A significant decrease in the rate of repair of both *cis*-DDP and BCNU induced DNA interstrand cross-links, with a corresponding decrease in the clonogenic survival of the cells, was observed following novobiocin exposure. Although the peak cross-link indices of novobiocin-treated cells relative to controls were not significantly increased, residual DNA cross-linking in the cells after 72 h was increased by 1.4-fold for BCNU and 3-fold for cells treated with *cis*-DDP, thus, indicating a greater effect of topoisomerase II on cross-link repair than on cross-link formation. These data suggest that inhibition of topoisomerase II may provide a potentially effective clinical strategy for sensitizing human brain tumors, and possibly other tumors as well, to DNA cross-linking anticancer agents.

INTRODUCTION

The 2-chloroethylnitrosoureas and the platinum analogue, *cis*-DDP[3] are agents with proven clinical activity against human malignant gliomas (1, 2). The antitumor action of both classes of agents is related to their ability to induce multiple intracellular lesions that impair the processes of gene transcription and translation, as well as with protein function. DNA interstrand cross-links in the cellular genome have now been shown to be among the most cytotoxic of the lesions induced by these agents (3–6). Unrepaired, the cross-links inhibit normal DNA replication and transcription and ultimately result in cell death. Although the complex molecular mechanisms involved in the repair of drug damaged DNA are still the subject of much investigation, there is evidence to suggest a role for topoisomerase II in the repair process (7–12). Topoisomerase II is a major nonhistone protein that is associated with the nuclear scaffold and is present at the base of nucleosomes (13–15). It participates in the structural organization of chromatin, in DNA replication and transcription, and in cell proliferation (13–20). The function of topoisomerase II in these cellular events is based, in part, on its ability to relax DNA in a two-step process involving nicking both strands of the DNA double helix and religating the strands (13–15). As a result, topoisomerase II has been targeted in the development of anticancer agents that act by inhibiting its function (21, 22). The change in DNA topology induced by topoisomerase II can potentially affect the availability of DNA sequences for alkylation or platination, and for subsequent repair of the damaged DNA. In this study, we investigated the inhibition of topoisomerase II activity in a human glioblastoma multiforme cell line, and examined how this inhibition modulated the kinetics of both the formation and the repair of DNA ISCs in the total genome, as well as the sensitivity of the tumor cells to *cis*-DDP and BCNU. Inhibition of topoisomerase II activity was achieved in these studies with novobiocin, a nalidixic acid analogue which is also a known potent inhibitor of topoisomerase II, at concentrations that were noncytotoxic to the tumor cells.

MATERIALS AND METHODS

Biochemicals. BCNU was purchased from Bristol-Myers Squibb Co., Wallingford, CT. Stock solutions were made in minimal ethanol and diluted to the desired working concentrations with Hanks' buffered salt solution. *cis*-DDP was purchased in aqueous solution and diluted just before use. RNase A and proteinase K were purchased from Boehringer-Mannheim, Frankfurt, Germany.

Glioblastoma Multiforme Cell Line. The HBT 28 cell line used in this study was established in our laboratory from a fresh tumor biopsy, as previously described (23). The tumor was classified neuropathologically as a glioblastoma multiforme. The neoplastic nature of cells of the resulting cell line was confirmed by cytomorphology after hematoxylin/eosin staining and their astrocytic origin by avidin-biotin immunoperoxidase staining for the astrocyte-specific glial fibrillary acidic protein. Cells used in this study had undergone less than 10 *in vitro* passages and were maintained routinely as monolayers in Dulbecco's minimal essential medium supplemented with 15% fetal calf serum.

Preparation of Isolated Nuclei and Nuclear Extracts. Tumor cells (2.5×10^6) washed in phosphate-buffered saline were resuspended in 1 ml of 5 mM potassium phosphate buffer (pH 7.5) containing 2 mM $MgCl_2$, 1 mM phenylmethylsulfonyl fluoride, 1 mM 2-mercaptoethanol, 0.1 mM sodium EDTA, 0.5% Nonidet P-40. After 15 min at 37°C, the mixture was subjected to gentle mechanical disruption and centrifuged at $300 \times g$ for 10 min. The pellet was then washed once in one-half the original suspension volume of 5 mM potassium phosphate buffer (pH 7.5), containing 350 mM NaCl, 0.5 mM phenylmethylsulfonyl fluoride, and 10 mM 2-mercaptoethanol, and the isolated nuclei were recovered by centrifugation at $1000 \times g$ for 10 min. The isolated nuclei were homogenized in 250 μl of 25 mM Tris-HCl (pH 7.8) containing 6 mM $MgCl_2$, 1 mM dithiothreitol, 0.2 mM KCl, and 10% glycerol. The mixture was centrifuged at $10,000 \times g$ at 4°C for 15 min, and the supernatant (nuclear extract) was recovered. Protein content was determined by the method of Lowry *et al.* (24).

DNA Topoisomerase II Assay. This was determined as the ability of topoisomerase II to decatenate highly catenated K-DNA, using a modification of

Received 5/26/93; accepted 9/22/93.

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[1] This research was supported by Grants CA 46410 and CA 555835 from the National Cancer Institute, NIH, and Grant 100-R104 from the Bristol-Myers Squibb Company.

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[3] The abbreviations used are: *cis*-DDP, *cis*-diammine, dichloroplatinum (II); BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; ISC, interstrand cross-link; K-DNA, kinetoplast DNA.

a previously described assay (25). ^{3}H-Labeled K-DNA was isolated from *Crithidia fasciculata* cultures grown in the presence of 10 μCi/ml of [^{3}H]-thymidine and purified on a CsCl gradient as previously described (26, 27). Tumor cell nuclear extract (25–50 μl; 25 μg) was incubated with 0.5 μg K-DNA in 10 mM Tris-HCl (pH 7.6), 5 mM $MgCl_2$, 50 mM KCl, 5 mM ATP, 1 mM dithiothreitol at 37°C for 30 min. The reaction was stopped with 10% sodium dodecyl-sulfate and 0.05% bromphenol blue and the sample was electrophoresed in a 1% agarose gel. The 2.5-kbp bands of decatenated K-DNA was cut out, solubilized in 10% $HClO_4$, and the radioactivity was counted by β scintillation. Nuclear extracts of L1210 nuclear stored at −70°C and bovine serum albumin were used as positive and negative controls, simultaneously. The optimum amount of nuclear extract protein required for the topoisomerase assay was determined by performing the decatenation assay with up to 100 μg nuclear extract protein in the assay mix.

Assay of Tumor Cell Sensitivity to BCNU, cis-DDP, and Novobiocin. The capillary tumor clonogenic cell assay (28) was used to determine tumor cell sensitivity to BCNU and *cis*-DDP, both alone and in combination with novobiocin, and for determining the nontoxic concentration of novobiocin for the modulation studies. Cell cultures in late exponential growth were trypsinized, washed, and resuspended at 2.5×10^4 cells/ml in a cloning mixture consisting of enriched CMRL 1066 medium (28) and 0.2% agarose, and 0–200 μM novobiocin, 0–100 μM BCNU, or 0–10 μM *cis*-DDP. The mixture was vortexed and 30 μl were drawn into triplicate sterile glass capillary tubes. After gelling, the tubes were incubated in a humidified atmosphere at 37°C and 5% CO_2. Two weeks later, the agarose was flushed out of the capillary tubes onto glass slides, and colonies (50 μm or greater in size) were counted under low-power phase contrast microscopy. The surviving fraction of the clonogenic cells at each drug concentration was determined and survival curves were constructed by plotting log surviving fractions against the drug concentrations.

Assay of Cellular DNA Interstrand Cross-Linking. Total cellular genomic DNA interstrand cross-linking was quantitated using an ethidium bromide DNA-binding method, developed from the one we previously described (29). The novel aspects of this modified DNA ISC assay were the elimination of S_1 nuclease digestion and dialysis of the isolated DNA, and the measurement of the ethidium bromide-DNA complex at an excitation wavelength of 305 nm instead of 525 nm, as in previous assays. The shorter higher energy excitation wavelength, determined by prior scanning spectrofluorometry, resulted in an increase in the sensitivity of cross-link detection by almost 2-fold. For this assay, 2.5×10^6 tumor cells were lysed in 0.5% sodium dodecyl sulfate. RNA and protein were digested by treating the cell lysates with 100 μg/ml DNase-free RNase A followed by 1 mg/ml proteinase K for 1 h at 50°C. The DNA was precipitated by adding 50 μl of 20 mM ammonium acetate and 2.5 volumes of ethanol and washed twice with 70% ethanol. With this modification, assay time was reduced by almost a full day, while maintaining the yield of high molecular weight cellular DNA (approximately 80 kilobase pairs by agarose gel electrophoresis) with the same level of purity as with the previous method. Subsequent steps of the cross-link measurement were as we previously described (29). Briefly, to each set of triplicate 100-μl (5–10 μg) aliquots of isolated DNA in TE buffer was added 0.9 ml of 20 mM potassium phosphate and 2 mM EDTA, pH 11.75. One set of tubes was heated at 100°C for 10 min in a heating block (Dribath-Thermolyne, Dubuque, IA) and cooled at room temperature for 5 min. The heated and unheated tubes were equilibrated at 15°C for 10 min and ethidium bromide was added to 1 μg/ml in a final volume of 3 ml. The fluorescence was then measured at an excitation wavelength of 305 nm (slit width 3) and an emission wavelength of 585 nm (slit width 20) in an LS 50 variable wavelength spectrofluorometer (Perkin Elmer, Norwalk, CT).

Quantitation of DNA Cross-Link Index and Computation of Rate of Cross-Link Repair. The level of DNA interstrand cross-linking induced in cells after each drug treatment was determined as previously described (29, 30) and is based on the assumption of a Poisson distribution of interstrand cross-links in a constant size of DNA. The cross-link index, CLI, was defined as:

$$\mathrm{CLI} = \ln [x_0/x_d]/ - \ln x_0;$$

where x_0 and x_d are the respective fractional changes in DNA-ethidium bromide fluorescence of untreated cells and drug-treated cells.

The rate of DNA interstrand cross-link repair in the tumor cells was defined as the decrease in the CLI over time (from time point of peak cross-link index) under the experimental conditions. Repair of cross-links fitted a first order kinetic model and thus could be described by the equation:

$$k_R \cdot \mathrm{CLI} = -d\mathrm{CLI}_0/dt,$$

which upon integration gives:

$$\mathrm{CLI}_t = \mathrm{CLI}_0 \cdot e^{-k_R t}$$

where CLI_0 is the peak cross-link index and k_R is the rate of cross-link repair. By rearrangement and taking logs, k_R can be computed as:

$$k_R = \ln[\mathrm{CLI}_0/\mathrm{CLI}_t]/t$$

We examined whether during the 72 h over which DNA repair was measured, *de novo* synthesized DNA could dilute out the cross-linked DNA and thereby result in a decreased cross-link index that can be misinterpreted as DNA repair. For this, triplicate sets of cultures were treated with 25 μM *cis*-DDP or 50 μM BCNU for 2 h, rinsed, and incubated in fresh medium. Every 24 h, over 3 days, one set of cultures was trypsinized, a total cell count was performed, and the DNA was extracted and quantitated, as described earlier.

Modulation of Cellular DNA Cross-Link Formation and Repair by Novobiocin. Tumor cells were seeded at a density of 1×10^6 cells/T75 flask and allowed to grow to near confluency. Stock novobiocin was then added to achieve a 200 μM concentration. After 24 h, *cis*-DDP and BCNU were added to achieve final culture concentrations of 25 and 50 μM, respectively. The cultures were incubated for 2 h, rinsed, and postincubated in fresh medium containing 200 μM novobiocin for 0 to 72 h. Control cultures were similarly treated but without novobiocin. The DNA ISCs for both control cultures and cultures with novobiocin treatment were then determined over time as described earlier. The computed cross-link indices were plotted against time, and the rates of cross-link repair were computed as described earlier.

Effect of Novobiocin on Tumor Sensitivity to *cis*-DDP and BCNU. Tumor cells in late exponential phase growth, with and without prior exposure to 200 μM novobiocin for 24 h, were harvested and used to set up cloning mixtures containing *cis*-DDP (0–5 μM) and BCNU (0–100 μM). In the assays with cells that had been pretreated with novobiocin, novobiocin was again added to a final concentration of 200 μM. The mixtures were used to set up the capillary clonogenic cell assays as described earlier. The surviving fractions of cells, with and without novobiocin exposure, were computed and plotted against the *cis*-DDP and BCNU concentrations.

RESULTS

Topoisomerase II Activity and Its Inhibition by Novobiocin. The results of the kinetoplast decatenation assay for topoisomerase II activity are shown in Fig. 1. Fig. 1*a* was used to determine the optimum amount of nuclear extract protein required for the topoisomerase II inhibition study. As shown in Fig. 1*a*, linearity in enzyme activity was observed with increasing nuclear extract protein, up to 25 μg protein/assay. The effect of increasing novobiocin concentrations on the K-DNA decatenating activity of 25 μg nuclear extract, after a 1-h incubation, is shown in the dose-response curve in Fig. 1*b* and the agarose gel electrophoresis in Fig. 1*c*. The inhibition of the decatenation (topoisomerase II) activity by novobiocin was dose dependent, with a maximum of 73% (27% of control) at 200 μM novobiocin.

Effect of Novobiocin on DNA Interstrand Cross-Link Repair. Table 1 and Fig. 2 summarize the results of the studies on the effects of novobiocin on the repair of DNA ISCs following exposure of HBT28 cells to 25 μM *cis*-DDP and 50 μM BCNU. Although novobiocin did not significantly alter the peak level of *cis*-DDP- and BCNU-induced DNA ISCs, there was a significant alteration in the rate at which the cross-links were repaired. For BCNU, DNA ISCs were repaired at the rate of 1.49×10^{-2}/hr in control compared to 1.03×0^{-2}/h in novobiocin-treated cells. A more profound effect of novobiocin on DNA ISC repair was observed for *cis*-DDP-treated cells. The DNA ISC repair rates were $9.17 \times 10^{-2} \cdot \mathrm{hr}^{-1}$ for control and 1.27×10^{-2}/h for novobiocin-treated cells. The level of residual *cis*-DDP-induced cross-link, at 72 h post-*cis*-DDP treatment, was 3-fold in

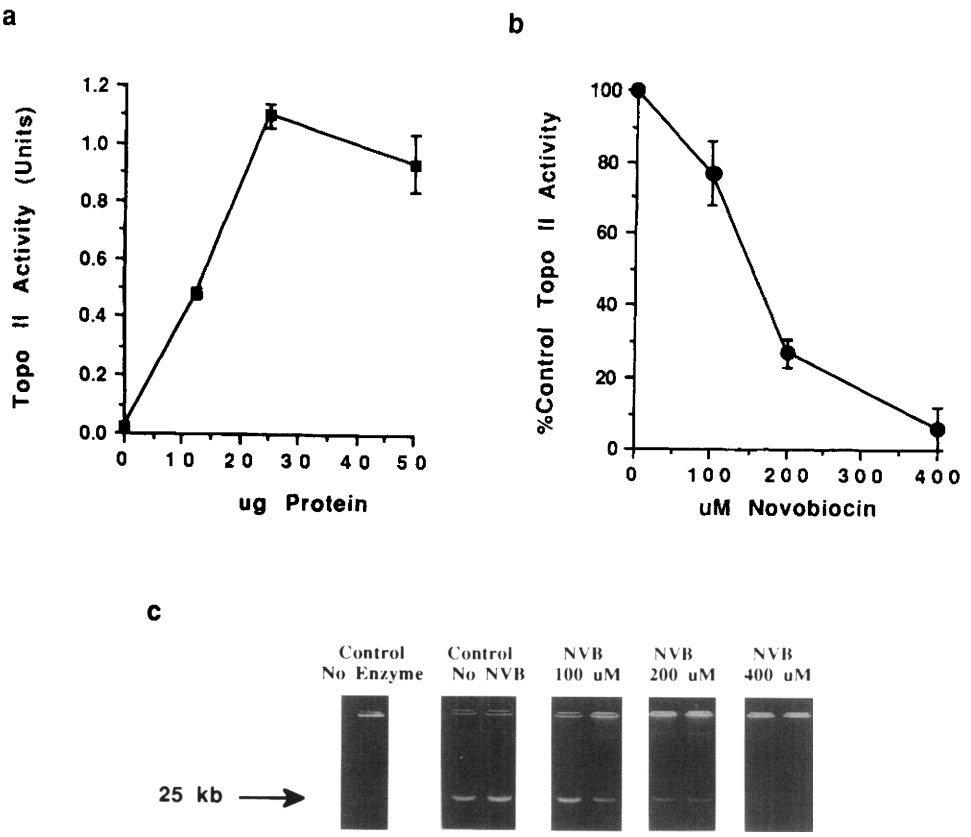

Fig. 1. *a*, relationship between concentration of HBT 28 nuclear protein (μg/ml) and topoisomerase II activity; *b*, dose-response curve for inhibition of topoisomerase II activity in HBT 28 nuclear extract by novobiocin; *c*, agarose gel electrophoresis of K-DNA treated with nuclear extracts of HBT 28 cells that had been incubated with increasing concentrations of novobiocin (*NVB*).

novobiocin-treated cells relative to controls. In contrast, residual BCNU-induced cross-linking was approximately 1.5-fold higher in novobiocin-treated cells than in controls. The results of the study to determine whether *de novo* DNA synthesis affected the DNA repair data are shown in Table 2. The number of cells in cultures treated with both *cis*-DDP and BCNU were essentially the same over the first 48 h following drug treatment, with only a slight decrease after 72 h, relative to controls. The total DNA content of the drug-treated cultures showed less variation over the 3-day period. For cells treated with 50 μm BCNU and 25 μm *cis*-DDP, the amount of total DNA isolated per culture was 0.30 μg and 0.33 μg, respectively, at the beginning of post-drug incubation and 0.24 μg and 0.26 μg, respectively, after 72 h.

Novobiocin Modulation of *cis*-DDP and BCNU Sensitivity of Tumor Cells. The *cis*-DDP and BCNU survival curves of the tumor cells, with and without novobiocin, are shown in Fig. 3. At 5 mM *cis*-DDP, the log surviving fractions of control cells and cells treated with 200 mM novobiocin were 0.48 and 0.10, respectively; for cells treated with 50 mM BCNU, the values were 0.29 and 0.098, respectively.

Table 1 *Effect of novobiocin on DNA interstrand cross-linking and survival of human glioblastoma cells treated with BCNU and cis-DDP*

Cells were treated with 200 μM novobiocin for 24 h before and following exposure to 50 μM BCNU and 25 μM *cis*-DDP (for cross-linking studies) and at 5 μM *cis*-DDP and 50 μM BCNU (for survival determination). The rate of DNA ISC repair, K_R, was expressed as CLI-h. All data were expressed as mean ± SD.

	Control		Novobiocin-treated	
	BCNU	*cis*-DDP	BCNU	*cis*-DDP
Peak DNA cross-link index	0.61 ± 0.04	1.10 ± 0.06	0.65 ± 0.05	1.33 ± 0.07
k_R (CLI/h) × 10^{-2}	1.49 ± 0.05	9.17 ± 0.09	1.03 ± 0.03	1.27 ± 0.04
Residual cross-link after 72 h	0.25 ± 0.01	0.21 ± 0.02	0.35 ± 0.04	0.62 ± 0.07
Surviving fraction	0.30 ± 0.09	0.43 ± 0.02	0.12 ± 0.01	0.04 ± 0.003

DISCUSSION

A major factor underlying the failure of chemotherapy in malignant gliomas is tumor resistance to the two classes of drugs most widely used to treat these tumors, namely, the 2-chloroethyl-nitrosoureas and the platinum analogues. For these agents, it has now been clearly established that a correlation exists between cytotoxicity and the level of DNA ISCs they induce in tumor cells (3–6). Recently, we reported that human malignant glioma cells could effectively repair DNA interstrand cross-links induced in their genome by both BCNU and *cis*-DDP (31). In the present study, we investigated this further by examining the role that the nuclear protein, topoisomerase II, might play in the repair of DNA ISCs induced in cells of a glioblastoma multiforme cell line by both BCNU and *cis*-DDP. Topoisomerase II, a chromatin-associated protein, has the unique function of altering DNA topology in a process that involves the formation of a topoisomerase II-DNA complex with the two strands of the DNA double helix, hydrolysis of the phosphodiester bond to yield a strand break, passing the two DNA strands through the gap and religating them (13–15). This action of topoisomerase II results in unwinding of the DNA and is critically important for DNA replication. We postulate that the accompanying topological change in the DNA, by making specific DNA segments more open, will affect not only the extent and, possibly, the site of DNA damage, but also, the accessibility of the damaged DNA to proteins involved in the repair of the damaged DNA. The enhanced repair would increase the survival of the tumor cells to cis-DDP and BCNU. Indeed, several reports (7–12, 31) have shown that DNA damage induced in cells by various physical and chemical agents can be inhibited by inhibitors of topoisomerase II inhibitors. It has also been reported that cycling cells, in which topoisomerase II activity is significantly increased, especially in the S phase, are less sensitive to BCNU than noncycling cells (32). Other reports have further indicated that tumor cell sensitivity to DNA

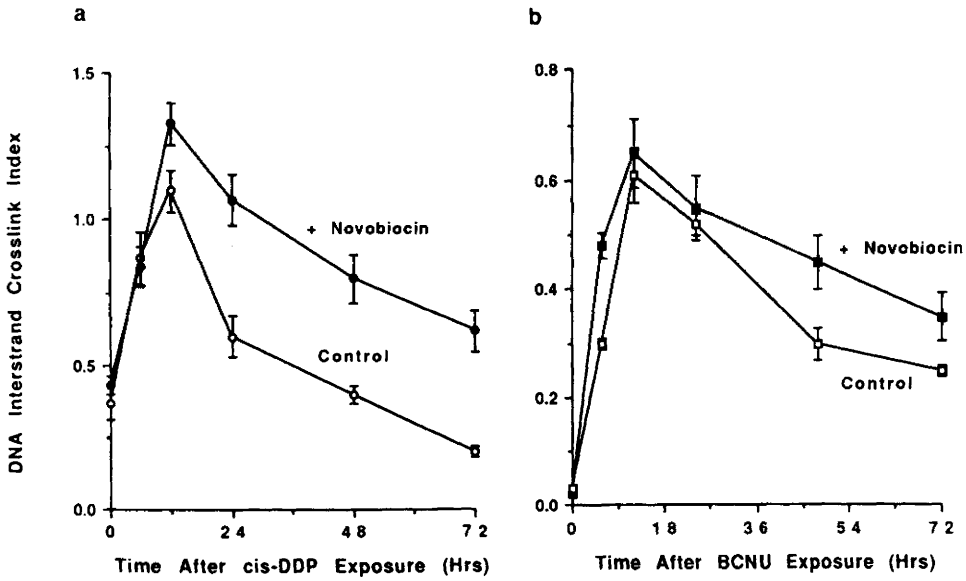

Fig. 2. Effect of 200 μM novobiocin on kinetics of formation and repair of DNA interstrand cross-links induced by (*a*) *cis*-DDP, and (*b*) BCNU.

cross-linking agents is enhanced by prior exposure of the cells to DNA topoisomerase II inhibitors (33). In a nitrogen mustard-resistant human Burkitt lymphoma cell line, a decreased level of DNA ISCs and a 3-fold increase in topoisomerase II activity has been demonstrated relative to its sensitive counterpart (34).

Our results indicate that for both *cis*-DDP and BCNU, the repair of DNA ISCs was significantly reduced by preexposure to novobiocin, and that this was accompanied by a corresponding increase in the sensitivity of the tumor cells treated to the two agents. In the DNA repair studies we did not separate newly synthesized DNA from previously existing DNA before quantitating the level of cross-linking. This poses the potential possibility that dilution of the damaged DNA with *de novo* synthesized DNA could have occurred and been misinterpreted as DNA repair. Our data, however, indicate that over the 72-h period that DNA cross-link repair was measured, there was no significant change in the cell number or DNA content of the drug-treated cultures, suggesting that such a DNA dilution artifact resulting from newly synthesized DNA or cell proliferation did not have a significant effect on the DNA repair data.

Despite the relatively small increase in the peak BCNU- and *cis*-DDP-induced cross-link index observed with novobiocin treatment, the extent of residual DNA cross-linking after 72 h was increased by 1.4-fold for BCNU-treated cells and 3-fold for cells treated with *cis*-DDP. At 200 μM novobiocin, the concentration used to modulate the tumor cells, *in vitro* topoisomerase II activity was inhibited by 73%, when the optimum amount (25 μg) of nuclear extract was used in the assay. Although these results demonstrate that, *in vitro*, novobiocin can inhibit brain tumor topoisomerase II activity and thereby potentiate the cytotoxic action of both BCNU and *cis*-DDP, they do not indicate the level to which such topoisomerase II inhibition is achieved *in vivo*.

Table 2 *Effect of incubation time on total cell number and DNA content of BCNU- and cis-DDP-treated cultures of human glioblastoma cells*

Cells were treated with 50 μM BCNU or 25 μM *cis*-DDP for 2 h, rinsed, refed, and incubated with fresh medium. Every 24 h, a total cell count was performed and the total DNA content was determined.

	50 μM BCNU		25 μM *cis*-DDP	
Incubation time (h)	Total cell no./ culture ($\times 10^6$)	Total DNA/ culture (μg)	Total cell no./ culture ($\times 10^6$)	Total DNA/ culture (μg)
0	1.61 ± 0.11[a]	0.30 ± 0.02	1.65 ± 0.05	0.33 ± 0.07
24	1.50 ± 0.08	0.29 ± 0.06	1.62 ± 0.06	0.26 ± 0.06
48	1.55 ± 0.07	0.27 ± 0.02	1.63 ± 0.09	0.28 ± 0.05
72	1.47 ± 0.06	0.24 ± 0.02	1.55 ± 0.08	0.26 ± 0.07

[a] Mean ± SD.

The exact mechanisms underlying the potentiation of cytotoxicity by novobiocin, or indeed by topoisomerase II inhibition, remain to be clarified. Recently, it was shown that novobiocin treatment induced a G_1-S block in Chinese hamster ovary cells with an accompanying increase in sensitivity of the cells to 4-hydroperoxycyclophosphamide, a potent DNA alkylating agent, and Adriamycin, a known DNA intercalator and topoisomerase II inhibitor (35). This ability of novobiocin and other topoisomerase II inhibitors to cause cell cycle arrest and block progression of cells into the DNA synthesis (S) phase or through G_2 (35–39) phase of the cell cycle may represent an attempt of the damaged cells to repair the drug-induced DNA ISCs at this checkpoint. Various putative DNA repair-associated proteins, such as the products of the *p53* gene and the retinoblastoma gene have been shown to be elevated in cells at the G_1-S junction (40). However, more definitive studies will be required to further elucidate the role of cell cycle checkpoint arrest in mediating repair of *cis*-DDP- and BCNU-induced DNA ISCs in human brain tumors.

The interpretation of the results of the effect of novobiocin on DNA repair in brain tumor cells is further compounded by the fact that, in addition to the potent ability of novobiocin to inhibit topoisomerase II activity, a number of other effects of novobiocin on cells have been reported. These include the inhibition of the function of DNA and RNA polymerases, and of DNA ligase by novobiocin (41–47). In addition, novobiocin has been shown to interfere with mitochondrial function through a reduction in mitochondrial ATP:ADP ratio (48), although other evidence (49) suggests that the reduction in mitochondrial ATP may not be the mechanism of novobiocin-mediated inhibition of DNA repair. Although no evidence for it exists, the possibility can also not be ruled out that novobiocin may potentiate BCNU and *cis*-DDP by enhancing their intracellular uptake.

Despite the potential multiple effects of novobiocin on cellular processes, the data we present indicate that the potentiating effect of novobiocin on BCNU and *cis*-DDP cytotoxicity in brain tumor cells is, at least in part, the result of decreased repair of DNA interstrand cross-links induced by both agents in the cells. The demonstrated ability of novobiocin to inhibit topoisomerase II activity further suggests that topoisomerase II inhibition may constitute part of the mechanism for the decreased DNA repair capacity of the tumor cells.

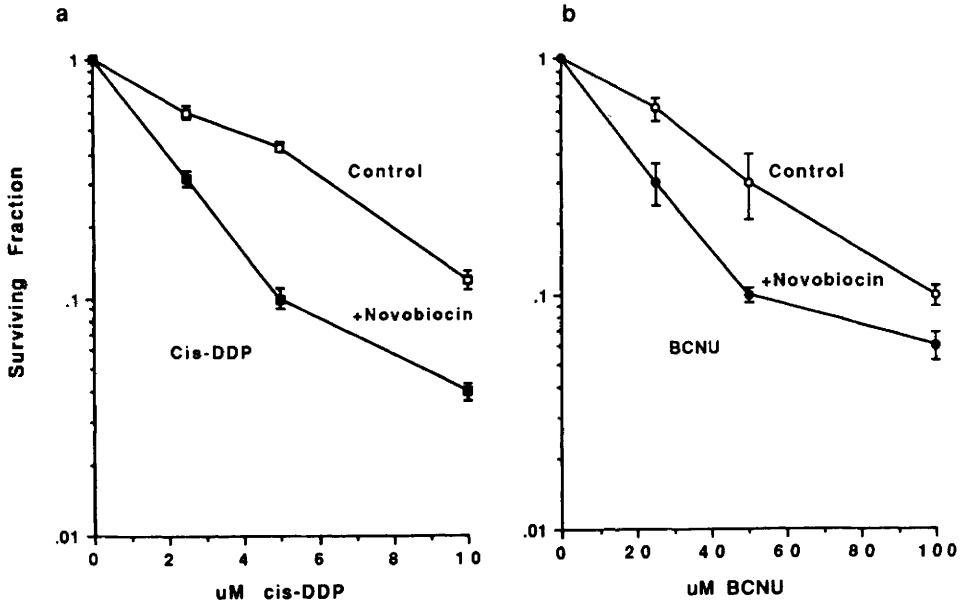

Fig. 3. Effect of 200 μM novobiocin on survival of HBT 28 cells treated with (*a*) *cis*-DDP, and (*b*) BCNU.

A previous clinical Phase I trial showed that the concentrations of novobiocin, at which the potentiation of alkylator cytotoxicity was observed in this study, may not be achievable systemically (50). In a subsequent Phase II trial of high dose cisplatin and p.o. novobiocin in non-small cell lung cancer (51), no significant advantage was observed over high dose *cis*-DDP alone. This could be explained, at least in part, by the fact that the levels of novobiocin measured in plasma of the patients were less than 50% of the novobiocin concentration at which significant enhancements of *cis*-DDP and BCNU cytotoxicity were observed in the present study. Combined, these data suggest that for this approach to be clinically feasible in brain tumor therapy, it will be necessary, as has been previously suggested (51), to have available novobiocin preparations, *e.g. i.v.* or intraarterial, that will result in levels in the central nervous system high enough to have a significant effect on DNA repair. Furthermore, the development of novel potent topoisomerase inhibitors with good blood-brain barrier penetrability may be required in order to exploit the full potential of topoisomerase II modulation in brain tumor alkylator therapy.

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