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3-Aminobenzamide and/or O^6-benzylguanine evaluated as an adjuvant to temozolomide or BCNU treatment in cell lines of variable mismatch repair status and O^6-alkylguanine-DNA alkyltransferase activity

SR Wedge, JK Porteous and ES Newlands

Department of Medical Oncology, Charing Cross Hospital, Fulham Palace Road, London W6 8RF, UK.

Summary O^6-benzylguanine (O^6-BG) and 3-aminobenzamide (3-AB) inhibit the DNA repair proteins O^6-alkylguanine-DNA alkyltransferase (AGT) and poly(ADP-ribose) polymerase (PARP) respectively. The effect of O^6-BG and/or 3-AB on temozolomide and 1,3-bis(2-chloroethyl)-nitrosourea (BCNU) cytotoxicity, was assessed in seven human tumour cell lines: six with an AGT activity of >80 fmol mg^{-1} protein (Mer^+) and one with an AGT activity of <3 fmol mg^{-1} protein (Mer^-). Three of the Mer^+ cell lines (LS174T, DLD1 and HCT116) were considered to exhibit resistance to methylation by a mismatch repair deficiency (MMR^-), each being known to exhibit microsatellite instability, and DLD1 and HCT116 having well-characterised defects in DNA mismatch binding. Potentiation was defined as the ratio between an IC_{50} achieved without and with a particular inhibitor treatment. Temozolomide or BCNU cytotoxicity was not potentiated by either inhibitor in the Mer^- cell line. Preincubation with O^6-BG (100 μM for 1 h) was found to potentiate the cytotoxicity of temozolomide by 1.35- to 1.57-fold in Mer^+/MMR^+ cells, but had no significant effect in Mer^+/MMR^- cells. In comparison, O^6-BG pretreatment enhanced BCNU cytotoxicity by 1.94- to 2.57-fold in all Mer^+ cell lines. Post-incubation with 3-AB (2 mM, 48 h) potentiated temozolomide by 1.35- to 1.59-fold in Mer^+/MMR^+ cells, and when combined with O^6-BG pretreatment produced an effect which was at least additive, enhancing cytotoxicity by 1.97- to 2.16-fold. 3-AB treatment also produced marked potentiation (2.20- to 3.12-fold) of temozolomide cytotoxicity in Mer^+/MMR^- cells. In contrast, 3-AB produced marginal potentiation of BCNU cytotoxicity in only three cell lines (1.19- to 1.35-fold), and did not enhance the cytotoxicity of BCNU with O^6-BG treatment in any cell line. These data suggest that the combination of an AGT and PARP inhibitor may have a therapeutic role in potentiating temozolomide activity, but that the inhibition of poly(ADP-ribosyl)ation has little effect on the cytotoxicity of BCNU.

Keywords: temozolomide; BCNU; O^6-benzylguanine; 3-aminobenzamide; mismatch repair deficiency

Temozolomide, a monofunctional methylating imidazotetrazinone, and BCNU, a bifunctional chloroethylnitrosourea, represent two different classes of chemotherapeutic alkylating agent. The anti-tumour activity of both compounds is dependent upon formation of a reactive alkyldiazonium ion (Weinkam and Lin, 1979; Denny *et al.*, 1994) and subsequent alkylation of the accessible nucleophilic atoms of DNA, predominantly N^7-guanine, followed by N^3-adenine and O^6-guanine (Roberts, 1978). Temozolomide cytotoxicity can largely be accredited to methylation of O^6-guanine (Domoradzki *et al.*, 1984; Margison and O'Connor, 1990) and N^3-adenine (Karran *et al.*, 1982); the cytotoxicity/mutagenicity of O^6-methylguanine being attributed to the induction of futile cycling in the long patch mismatch repair pathway, which results in prolonged DNA strand interruptions and the inhibition of subsequent replication (Ceccotti *et al.*, 1993; Karran *et al.*, 1993). In contrast, the cytotoxicity of BCNU correlates with formation of a 1-[N^3-deoxycytidyl]-2-[N^1-deoxyguanosinyl]-ethane DNA interstrand cross-link (Bodell *et al.*, 1985; Jiang *et al.*, 1989), which is produced by intramolecular rearrangement of an O^6-chloroethylguanine adduct (Tong *et al.*, 1982*a*).

Temozolomide has recently demonstrated promising clinical activity in the treatment of glioblastoma and melanoma (Newlands *et al.*, 1992; O'Reilly *et al.*, 1993), while BCNU is an established agent for the treatment of many malignancies, including glioma and lymphoma (Young *et al.*, 1971; Edwards *et al.*, 1980). Nevertheless, the activity of DNA-alkylating chemotherapy is frequently compromised by the development of resistance; a phenomenon often related to the DNA repair capacity of the tumour cell (Harris *et al.*, 1983; Ludlum, 1990). It is therefore possible that the inhibition of one or more DNA-repair processes, by appropriate pharmacological intervention, may circumvent such resistance. This approach has been most widely examined with inhibitors of AGT, a DNA-repair protein responsible for the stoichiometric removal of adducts produced at the O^6-position of guanine (Pegg, 1983; Tano *et al.*, 1990). O^6-guanine adduct removal irreversibly inactivates AGT, requiring *de novo* synthesis of the protein to restore activity (Pegg, 1990). AGT depletion with the substrate analogue O^6-BG has been found to increase the cytotoxicity of both temozolomide and BCNU *in vitro* (Dolan *et al.*, 1991; Zeller and Magull-Seltenreich, 1995; Wedge *et al.*, 1996*a*), and their anti-tumour activity in xenograft experiments (Dolan *et al.*, 1993; Felker *et al.*, 1993; Friedman *et al.*, 1995; Wedge *et al.*, 1996*b*). More importantly, the administration of O^6-BG has been shown to increase the therapeutic index of BCNU *in vivo* (Mitchell *et al.*, 1992; Gerson *et al.*, 1993). However, there may be a need to identify additional DNA-repair mechanisms which can be inhibited to further potentiate DNA-alkylating therapies. This may be particularly relevant to temozolomide, since a methylation-tolerant phenotype can develop from a deficiency in the methyl-directed mismatch repair pathway (Branch *et al.*, 1993; Kat *et al.*, 1993; Karran *et al.*, 1994), which fails to recognise O^6-methylguanine and produce DNA strand breakage. Thus, increasing the retention of O^6-methylguanine by treatment with O^6-BG would not be expected to potentiate temozolomide cytotoxicity in a mismatch repair-deficient cell line.

An additional target for manipulation may be PARP, an abundant chromatin-bound enzyme, which has been implicated as having a regulatory role in many cellular processes, including DNA repair (Satoh *et al.*, 1993). Unmodified PARP binds tightly to DNA strand breaks, but is released following auto-poly(ADP-ribosyl)ation (Satoh and Lindahl, 1992). It is suggested that this mechanism may function to prevent spurious transcription or recombination during

Correspondence: SR Wedge
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normal DNA metabolism, or may impede DNA synthesis to prevent replication on a damaged template (Satoh and Lindahl, 1992; Chatterjee and Berger, 1994). PARP may also have a role in nucleosomal unfolding, since it can ADP ribosylate and electrostatically displace histones (Althaus *et al.*, 1994; Buki *et al.*, 1995), which would relieve chromatin condensation and facilitate the accessibility of the strand interruption to DNA repair enzymes. Activation of PARP *in vitro* has been demonstrated following treatment with a number of methylating agents, including dimethyl sulphate (Durkacz *et al.*, 1980), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Juarez-Salinas *et al.*, 1979), methyl methanesulphonate (Satoh *et al.*, 1993), streptozotocin (Whish *et al.*, 1975) and temozolomide (Tisdale, 1985), while PARP inhibition with 3-AB has been found to enhance methylating cytotoxicity (Durkacz *et al.*, 1980; Bürkle *et al.*, 1987). PARP activation has also been implicated in response to treatment with a variety of DNA cross-linking agents, which include BCNU (Malapetsa *et al.*, 1995).

The effects of combining an AGT and PARP inhibitor, or inhibiting PARP in a mismatch repair-deficient cell line, have not been examined in relation to methylating agent cytotoxicity. In addition, relatively few studies have examined chloroethylnitrosourea cytotoxicity and PARP inhibition. The main aim of this study, therefore, was to determine the relative enhancement of temozolomide or BCNU cytotoxicity by treatment with O^6-BG and/or 3-AB in cell lines of varied AGT activity and mismatch repair status.

Materials and methods

Chemicals and drugs

Temozolomide was kindly supplied by Dr J Catino, Schering-Plough Research Institute, Kenilworth, NJ, USA, and BCNU was purchased from Bristol Myers Pharmaceuticals, Hounslow, Middlesex, UK. O^6-BG and the [^{3}H]methyl-labelled DNA substrate for the assay of AGT were generous gifts from Dr RC Moschel (NCI–Frederick Cancer Research and Development Center, Frederick, MD, USA) and Dr GP Margison (Paterson Institute for Cancer Research, Christie Hospital NHS Trust, Manchester, UK) respectively. All other chemicals were purchased from Sigma, Poole, UK.

Cell culture

Seven human cell lines were examined: four colorectal adenocarcinomas (Mawi, LS174T, HCT116 and DLD1), a breast adenocarcinoma (MCF-7), a malignant melanoma (StML-11a) and a glioblastoma astrocytoma (U87MG). Mawi was established at Charing Cross Hospital (Baer *et al.*, 1993), HCT116 and DLD1 obtained from Dr P Karran (Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Hertfordshire, UK), StML-11a from Dr C Zouboulis (Department of Dermatology, The Free University of Berlin, Germany), and all other cell lines from the European Tissue Culture Collection, Porton Down, UK. Cell lines were grown as monolayers in Dulbecco's modified Eagle medium (DMEM) or Roswell Park Memorial Institute 1640 tissue culture medium (RPMI-1640) (ICN Biomedicals, High Wycombe, UK), supplemented with 10% heat-inactivated fetal calf serum (FCS; Gibco, Paisley, UK), L-glutamine (2 mM), penicillin (100 U ml^{-1}) and streptomycin (100 μg ml^{-1}). Cultures were maintained in exponential growth at 37°C in a humidified 5% carbon dioxide incubator.

Assay of AGT activity

AGT activity was measured as removal of O^6-[^{3}H]methylguanine from a ^{3}H-methylated DNA substrate, as previously described (Lee *et al.*, 1991; Wedge *et al.*, 1996*a*). The AGT activity of an extract was expressed as fmol of [^{3}H]CH_3 transferred from the DNA substrate per mg of protein, using the assay of Bradford *et al.* (1976).

Cytotoxicity assay

Cytotoxicity was evaluated in 96-well plates as previously described (Wedge *et al.*, 1996*a*), using the sulphorhodamine-B (SRB) assay for protein (Skehan *et al.*, 1990). Conditions for O^6-BG incubation were chosen from AGT depletion data. 3-AB incubation conditions were selected from experiments in which 3-AB had been shown to inhibit DNA strand break rejoining and enchance methylating agent cytotoxicity (Durkacz *et al.*, 1980; Bürkle *et al.*, 1987). Both inhibitor treatments (alone and in combination) were determined to be non-growth inhibitory in each cell line. Briefly, cells were plated 24 h before a 1 h preincubation with 0.5% ethanol in DMEM or RPMI-1640, with/without O^6-BG (100 μM). Medium was then removed from all plates and replaced with that containing either temozolomide (3 h) or BCNU (1 h). Following drug incubation, medium was replenished with 0.2% dimethyl sulphoxide (DMSO) in DMEM or RPMI-1640 with/without 3-AB (2 mM). The medium of all plates was replaced with drug-free medium 48 h later, and plates reincubated for a further 5 days before SRB assay. IC_{50} values were interpolated by cubic spline regression. Potentiation of temozolomide or BCNU cytotoxicity by O^6-BG and/or 3-AB was taken to be the ratio between the IC_{50} achieved without inhibitor treatment divided by the IC_{50} achieved with inhibitor treatment.

Statistical analysis and evaluation of combination responses

The significance of a reduction in IC_{50}, obtained by pre- or post-incubation with a DNA repair inhibitor, was analysed using a one-tailed unpaired Student's *t*-test. The combined effect of O^6-BG and 3-AB on temozolomide cytotoxicity was evaluated using the multiple drug effect analysis of Chou and Talalay (1984), by treating 'temozolomide + O^6-BG' and 'temozolomide + 3-AB' as two separate 'drugs'. Dose–effect curves were constructed following each individual experiment for each 'drug' and for the combination in a fixed ratio (1 : 1) multiple dilution series, using the median effect equation (Chou, 1991). Computer software (Chou and Chou, 1987) was used to calculate a combination index (CI), with CI values of less than 1 being indicative of synergy, values of greater than 1 antagonism, and values equal to 1 additivity.

Results

AGT activity

The AGT activities of HCT-116 and DLD-1 were determined to be 87 ± 4.7 and 442 ± 17 fmol mg^{-1} protein respectively (mean $\pm$ s.e. from three independent samples). These activities were less than 10% of the control value (i.e. to 5.0 ± 0.7 and 9.9 ± 1.4 fmol mg^{-1} protein, respectively), 24 h after incubation with O^6-BG (100 μM, 1 h). Basal AGT activities of U87MG, StML-11a, LS174T, Mawi, and MCF-7 were previously determined to be 2.5, 113, 197, 535 and 721 fmol mg^{-1} protein respectively (Wedge *et al.*, 1996*a*). Depletion of AGT activity in these cell lines (by 100 μM O^6-BG for 1 h) was similar to that observed in DLD-1 and HCT-116.

Potentiation of cytotoxicity by O^6-BG and/or 3-AB

No significant enhancement of temozolomide or BCNU cytotoxicity was observed in U87MG, following either pretreatment with O^6-BG or post-treatment with 3-AB ($P > 0.1$, Tables I and II). The low AGT activity of this cell line combined with an inherent sensitivity to temozolomide alone, would correlate with a Mer$^-$/MMR$^+$ phenotype. The six remaining cell lines with intermediate/high AGT activities (Mer$^+$) were classified according to whether they were mismatch repair competent (MMR$^+$) or deficient (MMR$^-$). Those cell lines considered mismatch repair

Table I Cytotoxicity of temozolomide with or without O^6-BG and/or 3-AB

Phenotype	Cell line	Temozolomide IC_{50} (μM) Without O^6-BG or 3-AB	With O^6-BG	With 3-AB	With O^6-BG and 3-AB
Mer^-MMR^+	U87MG	11±2.0	9.8±0.9	11±2.6	10±1.6
Mer^+MMR^+	StML-11a	376±40	238±12	279±24	181±5.9
	Mawi	719±40	515±50	462±42	335±8.5
	MCF-7	801±27	599±36	583±43	403±41
Mer^+MMR^-	HCT116	502±31	489±46	225±25	187±27
	LS174T	1132±15	1068±29	531±7.5	508±22
	DLD1	1015±87	881±89	338±38	295±32

IC_{50} values were determined 7 days after a 3 h incubation with temozolomide, with/without O^6-BG pretreatment (100 μM, 1 h) and/or 3-AB post-treatment (2 mM, 48 h). Values represent the mean ± s.e. calculated from three separate experiments.

Table II Cytotoxicity of BCNU with or without O^6-BG and/or 3-AB

Phenotype	Cell line	BCNU IC_{50} (μM) Without O^6-BG or 3-AB	With O^6-BG	With 3-AB	With O^6-BG and 3-AB
Mer^-MMR^+	U87MG	53±4.3	47±2.9	57±1.3	59±2.7
Mer^+MMR^+	StML-11a	119±12	46±1.8	103±14	51±4.6
	Mawi	203±3.7	105±1.6	170±4.8	109±4.3
	MCF-7	192±15	92±5.1	157±6.2	79±6.7
Mer^+MMR^-	HCT116	65±1.2	28±3.9	46±2.3	23±1.7
	LS174T	159±4.5	65±5.4	143±11	69±7.9
	DLD1	141±10	62±0.6	132±6.6	59±1.6

IC_{50} values were determined 7 days after a 1 h incubation with BCNU, with/without O^6-BG pretreatment (100 μM, 1 h) and/or 3-AB post treatment (2 nM, 48 h). Values represent the mean ± s.e. calculated from three separate experiments.

deficient were DLD-1 (indistinguishable from HCT15) and HCT-116, which have mutations in the mismatch repair genes GTBP/p160 and hMLH1 respectively (Branch *et al.*, 1995; Drummond *et al.*, 1995), and LS174T, in which AGT depletion does not potentiate temozolomide cytotoxicity (Wedge *et al.*, 1996*a*), and which demonstrates microsatellite instability characteristic of a mismatch repair deficiency (Shibata *et al.*, 1994).

Temozolomide Pretreatment with O^6-BG significantly reduced the IC_{50} of temozolomide in Mer^+/MMR^+ cell lines ($P \leqslant 0.015$, Table I), and when 3-AB post-treatment was combined with O^6-BG pretreatment and temozolomide, it significantly increased the cytotoxicity of the O^6-BG and temozolomide combination ($P < 0.01$). Temozolomide cytotoxicity was enhanced to a similar extent by either O^6-BG pretreatment (1.35- to 1.57-fold) or 3-AB post-treatment (1.35- to 1.59-fold), and a combination of O^6-BG and 3-AB treatment enhanced temozolomide cytotoxicity by 1.97- to 2.16-fold (Figure 1a). CI values (mean ± s.d.) determined at the IC_{50} were 0.68±0.10 and 0.79±0.14 for mutually exclusive and non-exclusive combinations respectively, suggesting that the effect of combining an AGT and PARP inhibitor produces an approximately additive (if not slightly synergistic) enhancement of temozolomide cytotoxicity in Mer^+/MMR^+ cell lines.

In contrast, the IC_{50} of temozolomide in Mer^+/MMR^- cell lines was not reduced significantly by O^6-BG ($P > 0.05$), but very significantly reduced by 3-AB (Table I, $P \leqslant 0.0002$): treatment with 3-AB potentiating temozolomide cytotoxicity by 2.20–3.12-fold (Figure 1b).

BCNU O^6-BG pretreatment significantly reduced the IC_{50} of BCNU in both Mer^+/MMR^+ ($P < 0.003$) and Mer^+/MMR^- ($P < 0.001$) cell lines (Table II), thereby potentiating the cytotoxicity of BCNU by 1.94- to 2.57-fold (Figure 2). Post-treatment with 3-AB produced marginal potentiation (1.19- to 1.35-fold) of BCNU cytotoxicity in Mawi, MCF-7 and HCT116 (Figure 2). However, the addition of 3-AB to a combination of O^6-BG and BCNU did not significantly reduce the IC_{50} value obtained in any cell line ($P > 0.05$, Table II).

Discussion

While AGT depletion has been widely shown to potentiate the cytotoxicity of both methylating and bifunctional chloroethylating agents, studies investigating PARP inhibition and cytotoxicity have predominantly focused upon methylating agents such as dimethyl sulphate (Durkacz *et al.*, 1980), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Nduka *et al.*, 1980) and temozolomide (Boulton *et al.*, 1995). Although it has been suggested that the enhancement of BCNU cytotoxicity by the nucleoside analogue tiazofurin is related to the inhibition of PARP activity (Berger *et al.*, 1985), an additional study has indicated that the cytotoxicity of the bifunctional nitrosoureas is unaffected by PARP inhibition (Sebolt-Leopold and Scavone, 1992).

A wealth of experimental evidence suggests that there is some association between PARP activity and DNA strand breakage (Berger *et al.*, 1979; Durkacz *et al.*, 1980; Shall, 1984; Satoh and Lindahl, 1992). The mechanism by which 3-AB potentiates cytotoxicity could therefore be related to the alkylation of N^7-guanine and N^3-adenine by temozolomide (Bull, 1988) and BCNU (Tong *et al.*, 1982*b*), which can lead to DNA strand breakage following either enzymatic (Dianov and Lindahl, 1994) or spontaneous (Bailly and Verly, 1988) depurination. These strand breaks are likely to be bound by PARP (Satoh and Lindahl, 1992). The inhibition of PARP auto(ADP-ribosyl)ation would therefore impede the release of PARP from DNA (and/or nucleosomal unfolding), thereby preventing gap closure and potentiating the cytotoxicity of DNA-alkylating compounds.

A relationship between PARP and DNA strand breakage would support the findings of this study as follows:

(1) Temozolomide cytotoxicity in Mer^+/MMR^+ cells is dependent upon both the processing of O^6-methylguanine by a functional mismatch repair pathway and DNA strand breakage following depurination of 3-methyladenine and 7-methylguanine. These separate cytotoxic events can be independently enhanced by treatment with O^6-BG and 3-AB respectively, and an approximately additive response is obtained when the inhibitors are combined (Figure 1a).

(2) In Mer^+/MMR^- cells temozolomide cytotoxicity is

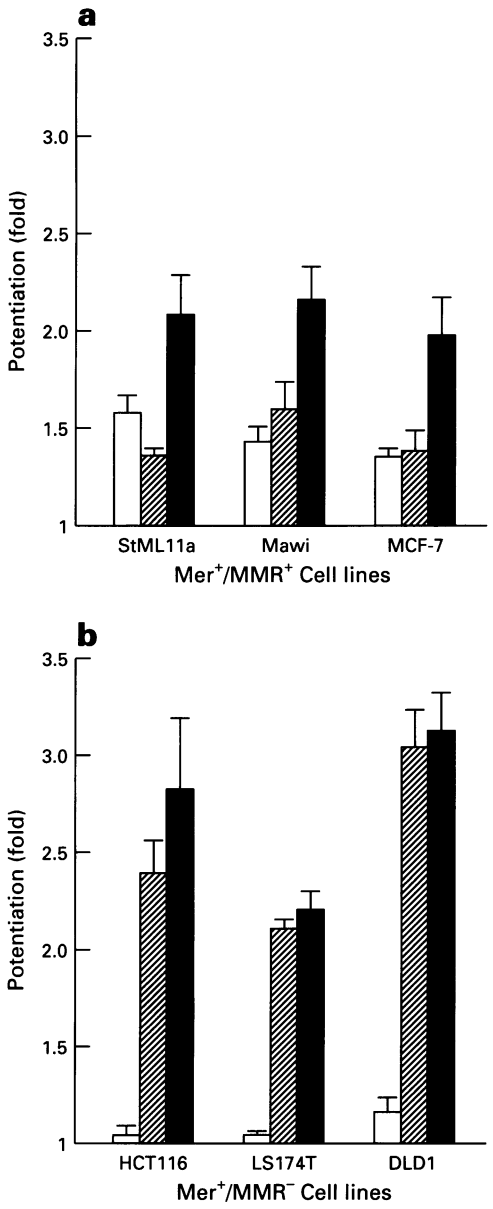

Figure 1 Potentiation of temozolomide cytotoxicity by O^6-BG pretreatment (□), 3-AB post-treatment (▨), or a combination of O^6-BG and 3-AB treatments (■), in (**a**) Mer^+/MMR^+ and (**b**) Mer^+/MMR^- cell lines. Cell lines within each group are arranged in order of increasing AGT activity. 'Potentiation' was defined as the ratio between an IC_{50} achieved without and with a particular inhibitor treatment. Each bar represents the mean potentiation ± s.e. from three independent experiments.

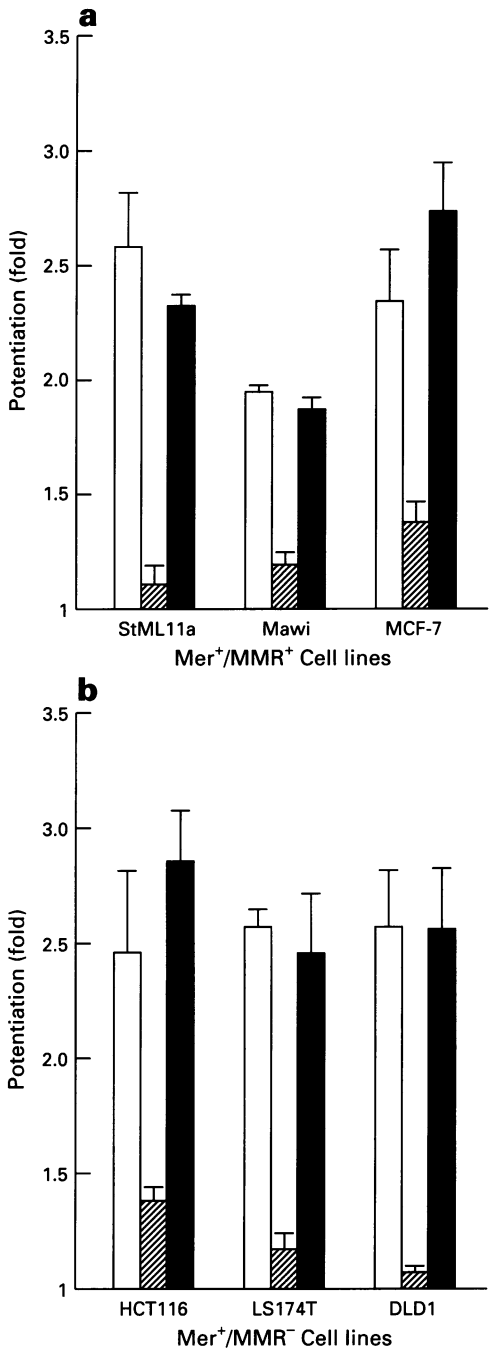

Figure 2 Potentiation of BCNU cytotoxicity by O^6-BG (□), 3-AB (▨), or a combination of O^6-BG and 3-AB (■). Symbols are as for Figure 1.

entirely dependent upon lesions resulting in strand breakage, and so only treatment with 3-AB can potentiate cell killing (Figure 1b).

(3) Any potentiation of BCNU cytotoxicity is independent of methyl-directed mismatch correction. The significant enhancement of BCNU cytotoxicity in Mer^+ cell lines following treatment with O^6-BG (Figure 2) is a consequence of increasing the number of highly toxic DNA interstrand cross-links (Lown *et al.*, 1978). Although PARP is known to bind to BCNU-damaged DNA (Malapetsa *et al.*, 1995), 3-AB produced little enhancement of BCNU cytotoxicity suggesting that DNA strand breakages arising from N^7-guanine and N^3-adenine adducts constitute only a minor effect towards BCNU cytotoxicity. This result supports the findings of Sebolt-Leopold and Scavone (1992).

(4) The lack of potentiation of either temozolomide or BCNU cytotoxicity by 3-AB in the U87MG Mer^-/MMR^+ cell line (Tables I and II) may suggest that DNA strand breakage also contributes little towards cell death in cells which are inherently very sensitive to DNA adducts produced at O^6-guanine.

These interpretations are complicated by a number of factors, not least that concentrations of 3-AB exceeding 1 mM can also inhibit mono(ADP-ribosyl) transferases (Rankin *et al.*, 1989), while even greater concentrations can induce perturbations in DNA precursor metabolism (Milam *et al.*, 1986). However, that treatment with 3-AB for 48 h did not inhibit the growth of any cell line (data not shown), and that the enhancement of temozolomide and BCNU cytotoxicity by 3-AB was profoundly different in the same cell line (e.g. DLD1, Figure 1 and 2), would suggest that the potentiation observed is unlikely to be related to an effect of 3-AB on DNA synthesis. An effect on ADP(ribose) metabolism is most probable, given that 3-AB (1 mM) can completely inhibit a

50% reduction in cellular NAD produced by treatment with 2 mM temozolomide (Boulton *et al.*, 1995). The association between DNA strand breakage and the inhibition of PARP with 3-AB, however, remains controversial (Boulton *et al.*, 1995), and it is possible that the enhancement of temozolomide cytotoxicity by 3-AB could be related to the inhibition of acceptor protein ADP-(ribosyl)ation, particularly if such proteins regulate cell cycle progression or apoptosis in response to DNA damage (Kastan *et al.*, 1991; Nosseri *et al.*, 1994; Malcomson *et al.*, 1995).

The magnitude by which methylation and chloroethylation cytotoxicity was enhanced by O^6-BG did not clearly correlate with AGT activity, although resistance to both agents can be multifactorial and dependent on factors other than at the level of DNA repair. These may include drug-detoxification mechanisms, involving glutathione-*S*-transferase (Smith *et al.*, 1989; Waxman, 1990) or metallothionine (Kelley *et al.*, 1988). In addition, the p53 injury–response pathway, which induces G_1 arrest and/or apoptosis in response to DNA damage (Kastan *et al.*, 1991; Malcomson *et al.*, 1995), may also contribute to drug resistance following mutational events or overexpression of *bcl*-2 (Miyashita and Reed, 1992; Fairburn *et al.*, 1994).

One concern with the use of DNA repair inhibitors is the increase in normal tissue toxicity from DNA-alkylating chemotherapy (Fairburn *et al.*, 1995), particularly the exacerbation of myelosuppression which is dose limiting for both BCNU and temozolomide. However, this may be clinically managed by appropriate bone marrow and haematopoietic support. The possible enhancement of mutagenesis by AGT depletion, particularly with a methylating agent (Yang *et al.*, 1994), should be treated with greater trepidation, although the potential short-term therapeutic gain may well outweigh this risk in patients with an otherwise dismal prognosis.

In conclusion, these data indicate that the inhibition of poly(ADP-ribosyl)ation has little effect on the cytotoxicity of BCNU, but that a combination of AGT and PARP inhibitor may have a useful therapuetic role in potentiating temozolomide activity. This study also emphasises that the use of a PARP inhibitor to enhance methylating agent activity will be unaffected by mismatch repair status. Further investigation of the concentration and schedule dependency of 3-AB as a potentiator of temozolomide cytotoxicity may therefore be warranted, although the clinical potential of PARP inhibition will only be realised if more potent and soluble inhibitors become available.

Abbreviations

Temozolomide, 8-carbamoyl-3-methylimidazo[5,1-*d*]-1,2,3,5-tetrazine-4(3*H*)-one, also known as NSC 362856, CCRG 81045 and SCH 52365; AGT, O^6-alkylguanine–DNA alkyltransferase (EC 2.1.1.63); PARP, poly(ADP-ribose) polymerase (EC 2.4.2.30); BCNU, 1,3-bis(2-chloroethyl)-nitrosourea (carmustine); O^6-BG, O^6-benzylguanine; 3-AB, 3-aminobenzamide.

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