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Poly(ADP–ribose) polymerase-independent potentiation of nitrosourea cytotoxicity by 3-aminobenzamide in human malignant glioma cells

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Abstract

Poly(ADP–ribose) polymerase is a zinc-finger DNA-binding protein that detects specifically DNA strand breaks generated by genotoxic agents and is thought to be involved in DNA repair. Here, we examined the effects of 3-aminobenzamide, a poly(ADP–ribose) polymerase inhibitor, on the chemosensitivity of human malignant glioma cells. 3-Aminobenzamide selectively potentiated the cytotoxicity of the nitrosoureas, nimustine, carmustine and lomustine in 10 of 12 human malignant glioma cell lines. In contrast, 3-aminobenzamide did not modulate the cytotoxic effects of doxorubicine, teniposide, vincristine, camptothecin or cytarabine. The nitrosoureas did not induce poly(ADP–ribose) polymerase activity in the glioma cells. Ectopic expression of truncated poly(ADP–ribose) polymerase containing the poly(ADP–ribose) polymerase DNA-binding domain, which acts as a dominant-negative mutant, in LN-18 or LN-229 cells did not alter the 3-aminobenzamide effect on nitrosourea-mediated cytotoxicity. Thus, 3-aminobenzamide may target another nicotinamide adenine dinucleotide (NAD)-requiring enzyme, but not poly(ADP–ribose) polymerase, when enhancing nitrosourea cytotoxicity in human malignant glioma cells. Carmustine cytotoxicity was associated with a G2/M arrest. Coexposure to carmustine and 3-aminobenzamide overcame this G2/M arrest in T98G cells, which are sensitized to carmustine by 3-aminobenzamide, but not in U251MG cells, which are refractory to 3-aminobenzamide-mediated sensitization to carmustine. Thus, 3-aminobenzamide-mediated sensitization to carmustine cytotoxicity may result from interference with the stable G2/M arrest response to carmustine in human glioma cells.

Keywords: Glioma; Poly(ADP–ribose) polymerase; Nitrosoureas; 3-Aminobenzamide

1. Introduction

Poly(ADP–ribose) polymerase is a chromatin-bound nuclear enzyme which is involved in numerous cellular processes including proliferation and replication, stress response, DNA repair and recombination. The enzyme is activated by exposure to agents that induce DNA single strand or double strand breaks. These are recognized by poly(ADP–ribose) polymerase by virtue of the aminoterminal DNA-binding zinc finger domain. Activation of poly(ADP–ribose) polymerase results in the transfer of ADP–ribose units from nicotinamide adenine dinucleotide (NAD) to a variety of nuclear acceptors, including histones, topoisomerases, lamins and SV40 large T antigen (Cleaver and Morgan, 1991). Since poly(ADP–ribose) polymerase contains a Asp–Glu–Val–Asp (DEVD) cleavage site within its DNA-binding domain and is processed during apoptosis in many cell types (Kaufmann et al., 1993), its possible role in apoptosis has been extensively studied. Different models of apoptosis using transformed or non-transformed cells have revealed different putative functions of poly(ADP–ribose) polymerase in the apoptotic pathway. Activation of poly(ADP–ribose) polymerase by ischemic brain injury or by neurotoxic agents like *N*-methyl-D-aspartate promotes depletion of cellular NAD and ATP, which results in energy depletion and apoptosis. Inhibitors of poly(ADP–ribose) polymerase may prevent apoptosis and as such are possible neuroprotective agents (Zhang et al., 1994). However, poly(ADP–ribose) polymerase is not required in all instances of apoptosis, since many types of apoptosis evolve unchanged in poly(ADP–ribose) polymerase-deficient fibroblasts and thymocytes (Leist et al., 1997; Wang et al., 1997).

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The role of poly(ADP–ribose) polymerase and of pharmacological or genetic poly(ADP–ribose) polymerase inhibition in chemotherapy-induced apoptosis has remained controversial. Loss of poly(ADP–ribose) polymerase activity has been reported to induce hypersensitivity to topoisomerase I inhibitors and resistance to topoisomerase II inhibitors in V79 hamster cells (Chatterjee et al., 1989). Similar results were obtained with HeLa cells depleted of poly(ADP–ribose) polymerase by antisense RNA (Ding et al., 1992; Ding and Smulson, 1994). These cells were not able to commence strand break rejoining of damaged DNA. However, DNA repair capacity was re-established at later time periods, indicating that the concentration of the enzyme in nuclei exceeded the requirement for DNA repair/replication (Ding et al., 1992). Poly(ADP–ribose) polymerase may be involved in the induction of p53 protein accumulation in response to genotoxic stress, whereas p53 function is unaltered in poly(ADP–ribose) polymerase-deficient cells (Whitacre et al., 1995; Agarwal et al., 1997). Cytoprotective effects of poly(ADP–ribose) polymerase are readily demonstrated in nonneoplastic cells of poly(ADP–ribose) polymerase$^{-/-}$ mice, which are highly sensitive to the alkylating agent, *N*-methyl-*N*-nitrosourea, or irradiation (Ménissier de Murcia et al., 1997). Here, we examined whether genetic or pharmacological poly(ADP–ribose) polymerase inhibition affects the chemosensitivity of human malignant glioma cells.

2. Materials and methods

2.1. Materials

Hygromycin B, nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate were purchased from Boehringer (Mannheim, Germany). Avidin biotin peroxidase complex came from Vector (Burlingame, CA). Doxorubicine, teniposide, cytarabine, nimustine and carmustine were provided by Bristol–Myers Squibb (Princeton, NJ). Lomustine was provided by Medac (Hamburg, Germany). Vincristine and camptothecin and all the other chemicals were obtained from Sigma (Deisenhofen, Germany). NAD-[adenine-2,8-^{3}H] dipotassium salt (specific activity: 30 Ci/mmol) was obtained from ICN (Irvine, CA).

2.2. Cell culture

Human glioma cell lines were maintained in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal calf serum, 1% glutamin and antibiotics. These cell lines have been characterized in previous studies (Weller et al., 1998). LN-18 and LN-229 transfectants expressing the aminoterminal poly(ADP–ribose) polymerase-DNA-binding domain peptide were obtained by electroporation with the pECV–poly(ADP–ribose) polymerase-DNA-binding domain vector, kindly provided by J. Menissier de Murcia (Schreiber et al., 1995), using a gene pulser (Biorad, Munich, Germany). Cells were selected for 3 weeks after transfection with hygromycin B (250 μg/ml). Expression of the transfected poly(ADP–ribose) polymerase-DNA-binding domain transgene was confirmed by immunoblot analysis. Pooled transfected cells were compared with hygro control cells, which were transfected with the respective empty vector (pECV-control vector) lacking a cDNA insert.

2.3. Cytotoxicity assay

Drug cytotoxicity studies were performed in 96-well plates. Glioma cell survival or proliferation were assessed by crystal violet staining at the indicated times after drug exposure. The cell culture medium was removed and surviving cells were stained with 0.5% crystal violet in 20% methanol for 20 min at room temperature. The plates were washed extensively under running tap water and then air-dried, and optical density values were read in an ELISA reader at 550 nm after cell lysis in 0.1 M sodium citrate in 50% ethanol.

2.4. Poly(ADP–ribose) polymerase immunoblot analysis

Immunoblot studies were performed according to standard procedures as previously described (Weller et al., 1998). Anti-human poly(ADP–ribose) polymerase (rabbit polyclonal) was obtained from Boehringer. Alkaline phosphatase-conjugated anti-rabbit IgG (Sigma) was used for the detection of poly(ADP–ribose) polymerase and the poly(ADP–ribose) polymerase-DNA-binding domain moiety by nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate staining.

2.5. Poly(ADP–ribose) polymerase activity assay

Determination of poly(ADP–ribose) polymerase activity was assessed with minor modifications as described (Wright et al., 1996). Briefly, 5×10^6 were seeded in 6-well plates and allowed to attach for 24 h. After treatment, the cells were trypsinized and then centrifuged. The pellet was resuspended in 500 μl assay buffer (56 mM HEPES, pH 7.5, 28 mM KCl, 28 mM NaCl, 2 mM $MgCl_2$, 0.25 μCi [^{3}H]NAD (specific activity: 30 Ci/mmol)) containing 0.01% digitonin to permeabilize the cells. After incubation for 10 min at 37°C, proteins ribosylated with [^{3}H]NAD were precipitated with 0.5 ml 15% trichloroacetic acid on ice, pelleted at 13,000 rpm in a microfuge and washed 1–2 times with 0.5 ml 6% trichloroacetic acid. The pellet was solubilized with 0.5 ml 0.1 N NaOH and the radioactivity was measured after addition of 5 ml scintillation cocktail in a Wallac Liquid Scintillation Counter. Some cells were kept on ice during the time of poly(ADP–ribose) synthesis. These cells served as the background for this enzymatic assay.

2.6. Cell cycle analysis

The cells were treated as indicated, incubated with trypsin for 3 min at 37°C, harvested, washed and fixed with 70% ice-cold ethanol. The cells (10^6) were stained with propidium iodide (50 μg/ml in phosphate-buffered saline, containing 100 μg/ml RNase A), washed and subjected to flow cytometric analysis of DNA content using a Becton Dickinson FACScalibur cytometer. Results are presented as histograms of 10^4 cells.

3. Results

3.1. 3-Aminobenzamide selectively potentiates nitrosourea-mediated cytotoxicity of human malignant glioma cells

To investigate the modulation of drug cytotoxicity of glioma cells by 3-aminobenzamide, we first determined the effects of 3-aminobenzamide when administered alone. At a concentration of 1 mM, 3-aminobenzamide was devoid of any intrinsic effect on glioma cell viability and proliferation in 72-h continuous exposure assays in all 12 human malignant glioma cell lines included in this study (data not shown). Next, three cell lines, LN-18, LN-229 and T98G, were treated with various cancer chemotherapy drugs in the absence or presence of 3-aminobenzamide. 3-Aminobenzamide strongly potentiated carmustine-mediated cytotoxicity in all three cell lines, while the effects of doxorubicine, teniposide, vincristine, camptothecin or cytarabine were unaffected by 3-aminobenzamide. In contrast, the cytotoxicity of other nitrosoureas, nimustine and lomustine, was also augmented by co-exposure of the cells to 3-aminobenzamide. At equimolar concentrations, lomustine was the most potent nitrosourea among the three drugs tested. Table 1 summarizes the EC_{50} values for carmustine-induced cytotoxic cell death in the absence or presence of 3-aminobenzamide. Only two cell lines, U87MG and U251MG, were refractory to 3-aminobenzamide-mediated sensitization, as indicated by an enhancement ratio of 1. There was no correlation between the EC_{50} values for carmustine cytotoxicity and the 3-aminobenzamide enhancement ratios. In contrast, there was strong correlation between EC_{50} values with and without 3-aminobenzamide ($r = 0.96$, $p = 0.000001$). These two results indicate that carmustine-resistant glioma cell lines are as likely to be sensitized by 3-aminobenzamide as are carmustine-sensitive cell lines, as already indicated by the rather homogeneous distribution of enhancement ratios, which are all between 1 and 3.4. Further, using our previously published data (Weller et al., 1998), we determined that neither p53 genetic nor functional status nor total p53 levels correlated with carmustine cytotoxicity or the 3-aminobenzamide enhancement ratios (data not shown).

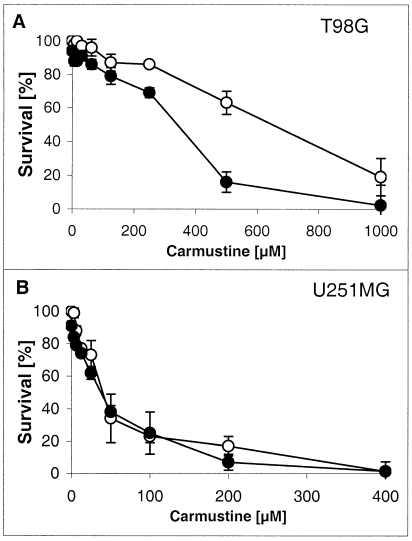

Fig. 1. Potentiation of carmustine cytotoxicity of glioma cells by 3-aminobenzamide. T98G (A) or U251MG (B) cells were exposed to increasing concentrations of carmustine in the absence (open symbols) or presence (filled symbols) of 3-aminobenzamide (1 mM) for 72 h. Viability was assessed by crystal violet staining. Data are expressed as mean percentages of survival and S.E.M. ($n = 3$).

Table 1
Potentiation of carmustine cytotoxicity of glioma cells by 3-aminobenzamide
EC_{50} values for carmustine cytotoxicity in the absence or presence of 3-aminobenzamide (1 mM) were obtained from experiments as shown in Fig. 1. The right outer column shows the enhancement ratio afforded by 3-aminobenzamide, expressed as EC_{50} without 3-aminobenzamide divided by EC_{50} with 3-aminobenzamide.

Cell line	EC_{50} carmustine (μM)	EC_{50} carmustine/3-aminobenzamide (μM)	3-Aminobenzamide enhancement ratio
LN-18	310±30	119±15	2.6
U138MG	220±20	137±25	1.6
U87MG	55±10	54±4	1
LN-428	320±20	139±18	2.3
D247MG	320±30	160±20	2
T98G	490±40	169±30	2.9
LN-319	900±80	346±40	2.6
LN-229	140±10	41±3	3.4
A172	460±50	219±30	2.1
U251MG	30±3	30±2	1
U373MG	440±30	244±20	1.8
LN-308	30±3	17±4	1.7

3.2. Carmustine does not activate poly(ADP–ribose) polymerase in human malignant glioma cells

Immunoblot analysis revealed that the poly(ADP–ribose) polymerase protein was expressed in all 12 cell lines. The lowest levels of poly(ADP–ribose) polymerase were detected in D247MG and U87MG cells (Fig. 2A). Thus, poly(ADP–ribose) polymerase levels appeared not to be linked to the 3-aminobenzamide enhancement ratios.

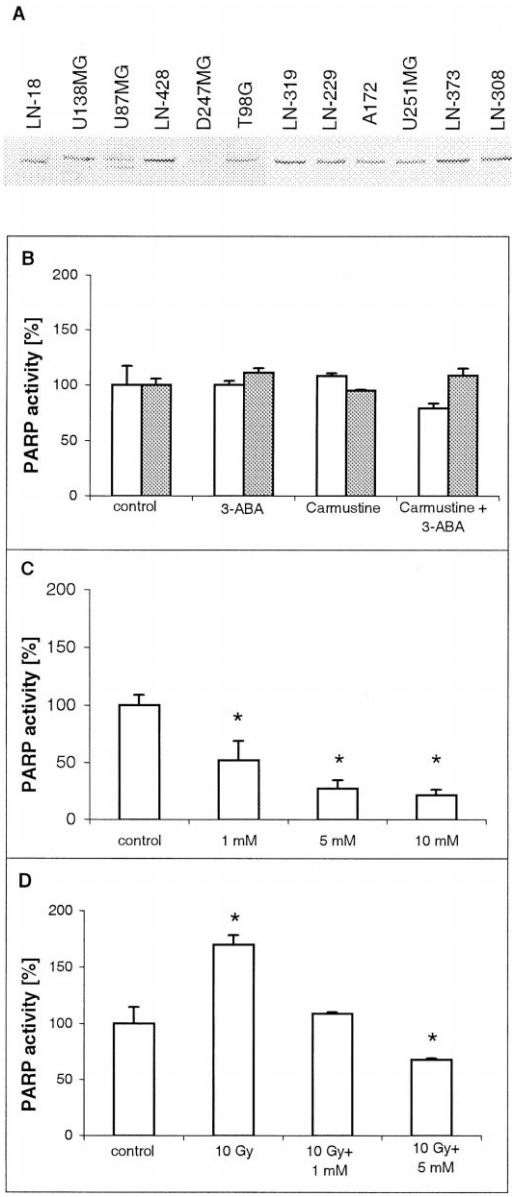

Fig. 2. Carmustine does not induce poly(ADP–ribose) polymerase activity in human malignant glioma cells. (A) Soluble lysates of the glioma cells were prepared and subjected to immunoblot analysis for poly(ADP–ribose) polymerase as described in Section 2. (B) LN-18 (open bars) or T98G (gray bars) cells were untreated or treated with 3-aminobenzamide (3-ABA, 1 mM) or carmustine (400 μM) or both. Poly(ADP–ribose) polymerase (PARP) activity was assessed at 1 h. (C) Jurkat T cells were untreated or treated with different concentrations of 3-aminobenzamide for 2 h. (D) Jurkat T cells were not pretreated or pretreated for 1.5 h with 3-aminobenzamide at 1 or 5 mM and then sham-irradiated or irradiated at 10 Gy, and then cultured in the absence or presence of 3-aminobenzamide at 1 or 5 mM. Poly(ADP–ribose) polymerase (PARP) activity was measured at 1 h after irradiation. Data in (B)–(D) are expressed as mean percentages and S.E.M. ($n = 3$). The 100% values correspond to 10,000 (T98G) and 8,000 cpm (LN-18) in (B), and in Jurkat T cells, to 809 cpm in (C), and 545 cpm in (D) ($^{*}P < 0.05$, *t*-test, compared with control cells).

We next asked whether poly(ADP–ribose) polymerase activity was modulated by nitrosoureas in glioma cells. LN-18 or T98G cells were exposed to carmustine (400 μM) in the absence or presence of 3-aminobenzamide, and poly(ADP–ribose) polymerase activity was determined 1 h later. The basal activity of poly(ADP–ribose) polymerase was neither augmented by exposure to carmustine nor reduced by 3-aminobenzamide (Fig. 2B). Carmustine had also no influence on poly(ADP–ribose) polymerase activity when applied for longer or shorter time intervals at various concentrations ranging from 100 to 1000 μM. In addition, 3-aminobenzamide did not inhibit the basal poly(ADP–ribose) polymerase activity even at a concentration of 5 mM (data not shown). As a positive control, Jurkat T cells were treated with increasing concentrations of 3-aminobenzamide. Fig. 2C shows that the basal poly(ADP–ribose) polymerase activity in Jurkat cells was inhibited by 3-aminobenzamide in a concentration-dependent manner. Further, poly(ADP–ribose) polymerase activ-

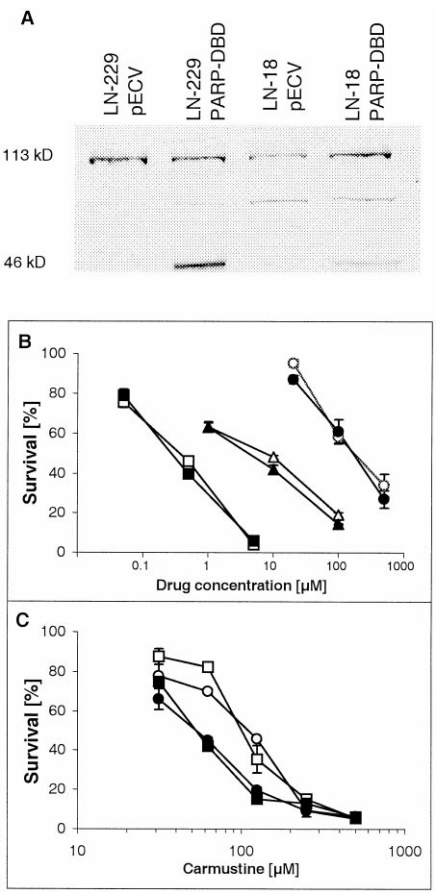

Fig. 3. Ectopic expression of the poly(ADP–ribose) polymerase-DNA-binding domain moiety fails to alter 3-aminobenzamide-induced augmentation of nitrosourea cytotoxicity. (A) Immunoblot analysis for full length poly(ADP–ribose) polymerase and the poly(ADP–ribose) polymerase-DNA-binding domain moiety (PARP-DBD) was performed as in Fig. 2 (for details, see Section 2). (B) Mock-transfected (open symbols) or poly(ADP–ribose) polymerase-DNA-binding domain-expressing (filled symbols) LN-229 cells were exposed to carmustine (circles), doxorubicin (squares) or teniposide (triangles) for 72 h. Data are expressed as in Fig. 1. (C) Mock-transfected (circles) or poly(ADP–ribose) polymerase-DNA-binding domain-expressing (squares) LN-229 cells were exposed to carmustine in the absence (open symbols) or presence (closed symbols) of 3-aminobenzamide (1 mM). Data are expressed as in Fig. 1.

ity was induced by irradiation in Jurkat T cells, and the irradiation-induced increase in poly(ADP–ribose) polymerase activity was inhibited by 3-aminobenzamide (Fig. 2D). In contrast, no increase in poly(ADP–ribose) polymerase activity was observed in 10 Gy-irradiated LN-229 cells (data not shown).

3.3. Ectopic expression of the dominant-negative poly(ADP–ribose) polymerase DNA-binding domain does not modulate 3-aminobenzamide-dependent sensitization of human glioma cells to nitrosourea cytotoxicity

The next set of experiments was conducted to further elucidate the role of poly(ADP–ribose) polymerase in the modulation of cytotoxicity by 3-aminobenzamide. To this end, LN-18 and LN-229 human malignant glioma cells were transfected with an expression plasmid coding for a 46 kDa polypeptide of the aminoterminal poly(ADP–ribose) polymerase DNA-binding domain, which acts as a dominant-negative poly(ADP–ribose) polymerase mutant (Schreiber et al., 1995). The expression of the poly(ADP–ribose) polymerase–DNA-binding domain moiety was confirmed by immunoblot analysis with a poly(ADP–ribose) polymerase-specific antibody that recognized both endogenous poly(ADP–ribose) polymerase at 113 kDa and the transgene product at 46 kDa (Fig. 3A). LN-18 or LN-229 cells engineered to express the poly(ADP–ribose)

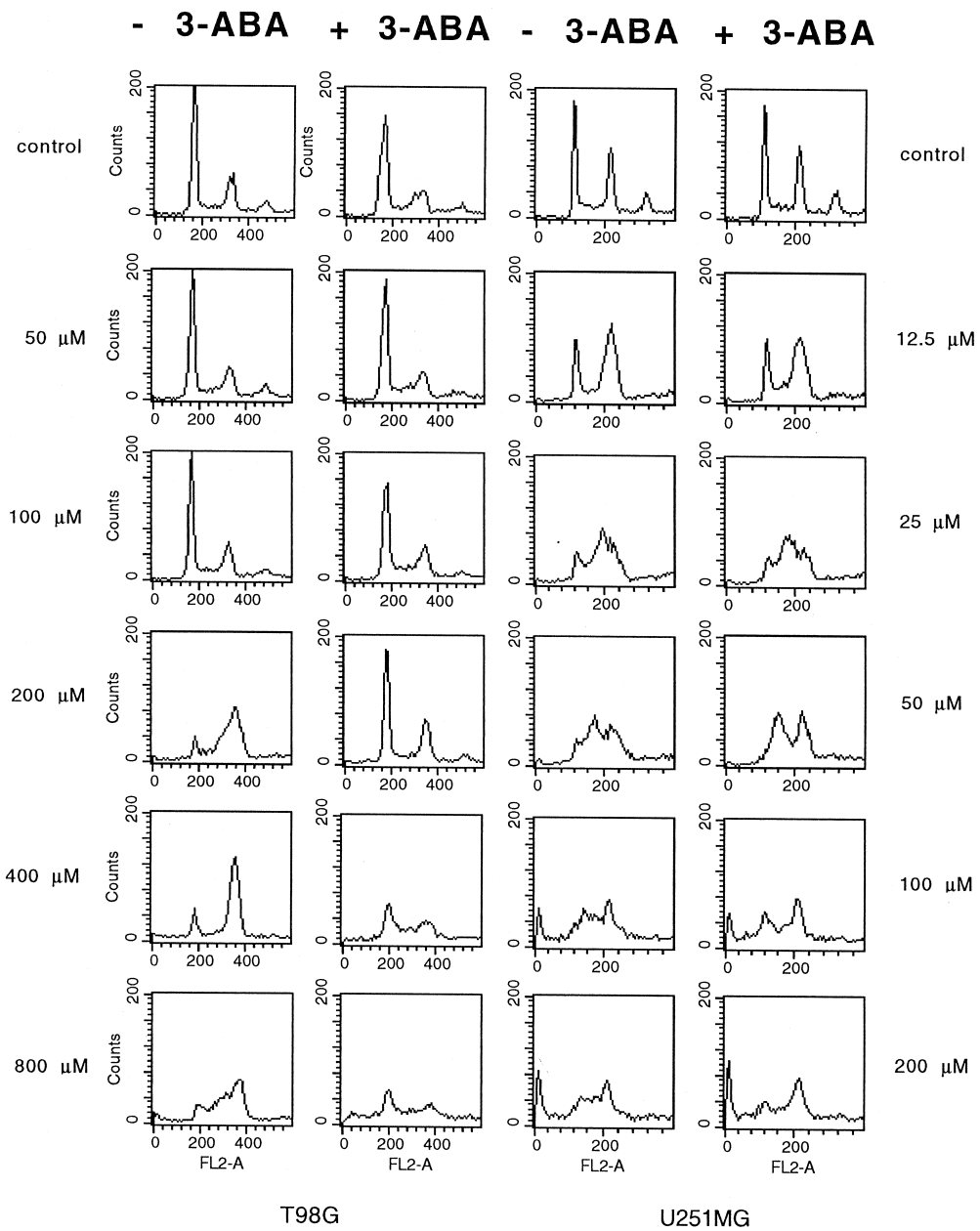

Fig. 4. 3-Aminobenzamide prevents carmustine-induced G2/M growth arrest. T98G (left) or U251MG (right) cells were untreated or treated with carmustine at various concentrations for 48 h in the absence or presence of 3-aminobenzamide (3-ABA, 1 mM) and analysed for cell cycle distribution by flow cytometry.

polymerase-DNA-binding domain moiety were no more sensitive towards nitrosoureas than the mock control transfectants. There were also no differences in their sensitivity to other drugs, as exemplified for doxorubicine or teniposide (Fig. 3B). When poly(ADP–ribose) polymerase-DNA-binding domain-expressing LN-229 cells were coincubated with nitrosoureas and 3-aminobenzamide, the cytotoxicity of carmustine and lomustine was enhanced in a concentration-dependent manner as it was in the mock-transfected control cells (Fig. 3C). The same result was obtained when poly(ADP–ribose) polymerase-DNA-binding domain-transfected LN-18 cells were incubated with nimustine, carmustine or lomustine in the presence of 1 mM 3-aminobenzamide for 72 h (data not shown).

3.4. 3-aminobenzamide overcomes the nitrosourea-induced G2/M arrest

Drug-induced glioma cell death is commonly associated with specific changes in cell cycle distribution (Winter and Weller, 1998; Borbé et al., 1999). Therefore, we next asked whether 3-aminobenzamide modulated the cell cycle changes induced by carmustine. Much higher concentrations of carmustine were required to induce G2/M accumulation in the more resistant T98G cell line than in the more sensitive U251MG cell line (Fig. 4, Table 2). Thus, G2/M accumulation was induced at 12.5 μM in U251MG cells but only at 200 μM in T98G cells. Coexposure to 3-aminobenzamide had little effect on carmustine-induced cell cycle changes in U251MG cells, corresponding to the negligible effect of 3-aminobenzamide on carmustine cytotoxicity (Table 1, Fig. 1). In contrast, coexposure to carmustine and 3-aminobenzamide in T98G cells, which induces much more cell death than carmustine alone (Table 1, Fig. 1), interfered with the G2/M arrest induced by exposure to carmustine alone, e.g., at 400 μM (Fig. 4) where there is prominent sensitization to cell death by 3-aminobenzamide (Fig. 1).

Table 2
Modulation of carmustine-induced cell cycle arrest by 3-aminobenzamide
The table provides quantitative data derived from experiments shown in Fig. 4. The sum of cells in G0/1, S and G2/M phase was normalized to 100%.

	Carmustine alone			Carmustine plus 3-aminobenzamide		
T98G	G0/1	S	G2/M	G0/1	S	G2/M
Control	64	6	30	66	7	27
50 μM	61	8	31	63	10	27
100 μM	60	15	25	55	11	34
200 μM	13	8	79	51	8	41
400 μM	14	6	80	35	21	44
800 μM	12	17	71	38	22	40
U251MG						
Control	40	18	42	41	18	41
12.5 μM	25	13	62	23	18	59
25 μM	16	33	51	12	44	44
50 μM	13	44	43	11	44	45
100 μM	27	23	50	28	26	46
200 μM	15	41	44	23	29	48

4. Discussion

Nitrosoureas have remained the cornerstone of chemotherapy for human malignant gliomas for the last 20 years. Their efficacy has been attributed to good blood brain and blood tumor barrier penetration and a cell cycle-independent mode of action.

Here, we report that the putative poly(ADP–ribose) polymerase inhibitor, 3-aminobenzamide, selectively sensitizes 10 of 12 human malignant glioma cell lines to the cytotoxic effects of nimustine, carmustine and lomustine (Table 1, Fig. 1). In contrast, 3-aminobenzamide does not modulate the cytotoxic effects of various other drugs with different modes of action, including doxorubicine, teniposide, vincristine, camptothecin or cytarabine. Previously, 3-aminobenzamide was shown to have negligible effects on carmustine cytotoxicity in various cancer cell lines (Wedge et al., 1996), but this study used different time schedules of carmustine and 3-aminobenzamide exposure. Although 3-aminobenzamide has been shown to modulate p53-mediated responses in glioma cells (Wang et al., 1998), at least 8 of 12 cell lines examined here lack wild-type p53 activity and, specifically, the abrogation of the carmustine-induced growth arrest in T98G cells (Fig. 4) was p53-independent since these cells are mutant for p53 (Weller et al., 1998). Interestingly, unlike radiosensitive Jurkat T cells, the radioresistant glioma cells did not exhibit increased activity of poly(ADP–ribose) polymerase in response to irradiation (Fig. 2D). It is tempting to speculate that the failure to up-regulate poly(ADP–ribose) polymerase activity may be radioprotective in glioma cells. Previous data on the radiosensitivity of cells from $PARP^{-/-}$ mice have been controversial (Ménissier de Murcia et al., 1997; Wang et al., 1997). Our data indicate that inhibition of poly(ADP–ribose) polymerase does not mediate 3-aminobenzamide-dependent sensitization of human malignant glioma cells to nitrosourea-induced cytotoxicity since poly(ADP–ribose) polymerase is not activated after exposure to nitrosoureas (Fig. 2), since 3-aminobenzamide does not modulate poly(ADP–ribose) polymerase activity at concentrations which enhance cell death (Figs. 1,2) and since the dominant-negative poly(ADP–ribose) polymerase-DNA-binding domain moiety does not alter the effects of 3-aminobenzamide on nitrosourea cytotoxicity (Fig. 3). These findings may add carmustine cytotoxicity of human glioma cells to the modes of apoptotic cell death that are not modulated by the absence or presence of poly(ADP–ribose) polymerase (Wang et al., 1997). Studies with more specific

poly(ADP–ribose) polymerase inhibitors may confirm that poly(ADP–ribose) polymerase is not involved in the potentiation of nitrosourea-mediated killing of human malignant glioma cells by 3-aminobenzamide. Inescapably, at present, we conclude that 3-aminobenzamide may act on other NAD-requiring enzymes to mediate the enhanced sensitivity to nitrosoureas in glioma cells.

Flow cytometric studies, comparing a 3-aminobenzamide-responsive cell line, T98G, and a 3-aminobenzamide-refractory cell line, U251MG, indicated that G2/M arrest and S phase accumulation were achieved at lower concentrations of carmustine in U251MG than in T98G cells (Fig. 4), consistent with the differential sensitivity of these cell lines to carmustine (Table 1). Interestingly, coexposure to carmustine and 3-aminobenzamide, which greatly enhanced cell death compared with carmustine treatment alone, prevented the G2/M accumulation and rather promoted cell cycle progression and S phase accumulation and, presumably, cell death out of S phase in T98G cells. No such effect was seen in U251MG cells (Fig. 4). Thus, 3-aminobenzamide may interfere with a stable, presumably cytoprotective G2/M arrest of glioma cells in response to carmustine treatment, which then results in enhanced cell death during continued efforts of the glioma cells to progress through the cell cycle. Interestingly, previous studies from our laboratory had failed to show a specific increase in cytotoxicity when similar studies were performed using classical G2/M abrogators such as caffeine or pentoxyfilline (Winter and Weller, 1998; Borbé et al., 1999).

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References

Agarwal, M.L., Agarwal, A., Taylor, W.R., Wang, Z.Q., Wagner, E.F., Stark, G.R., 1997. Defective induction but normal activation and function of p53 in mouse cells lacking poly-ADP–ribose polymerase. Oncogene 15, 1035–1041.

Borbé, R., Rieger, J., Weller, M., 1999. The failure of taxol-based combination chemotherapy for malignant glioma cannot be overcome by G2/M checkpoint abrogators or altering the p53 status. Cancer Chemother. Pharmacol. 44, 217–227.

Chatterjee, S., Cheng, M.F., Trivedi, D., Petzold, S.J., Berger, N.A., 1989. Camptothecin hypersensitivity in poly(adenosine diphosphate–ribose) polymerase-deficient cell lines. Cancer Commun. 1, 389–394.

Cleaver, J.E., Morgan, W.F., 1991. Poly(ADP–ribose) polymerase: a perplexing participant in cellular responses to DNA damage. Mutat. Res. 257, 1–18.

Ding, R., Smulson, M., 1994. Depletion of nuclear poly(ADP–ribose) polymerase by antisense RNA expression: influences on genomic stability, chromatin organization, and carcinogen cytotoxicity. Cancer Res. 54, 4627–4634.

Ding, R., Pommier, Y., Kang, V.H., Smulson, M., 1992. Depletion of poly(ADP–ribose) polymerase by antisense RNA expression results in a delay in DNA strand break rejoining. J. Biol. Chem. 267, 12804–12812.

Kaufmann, S.H., Desnoyers, S., Ottaviano, Y., Davidson, N.E., Poirier, G.G., 1993. Specific proteolytic cleavage of poly(ADP–ribose) polymerase: an early marker of chemotherapy-induced apoptosis. Cancer Res. 53, 3976–3985.

Leist, M., Single, B., Künstle, G., Volbracht, C., Hentze, H., Nicotera, P., 1997. Apoptosis in the absence of poly(ADP–ribose) polymerase. Biochem. Biophys. Res. Commun. 233, 518–522.

Ménissier de Murcia, J., Niedergang, C., Trucco, C., Ricoul, M., Dutrillaux, B., Mark, M., Oliver, F.J., Masson, M., Dierich, A., LeMeur, M., Walztinger, C., Chambon, P., De Murcia, G., 1997. Requirement of poly(ADP–ribose) polymerase in recovery from DNA damage in mice and in cells. Proc. Natl. Acad. Sci. U. S. A. 94, 7303–7307.

Schreiber, V., Hunting, D., Trucco, C., Gowans, B., Grunwald, D., De Murcia, G., Ménissier de Murcia, J., 1995. A dominant-negative mutant of poly(ADP–ribose) polymerase affects cell recovery, apoptosis, and sister chromatid exchange following DNA damage. Proc. Natl. Acad. Sci. U. S. A. 92, 4753–4757.

Wang, Z.Q., Stingl, L., Morrison, C., Jantsch, M., Los, M., Schulze-Osthoff, K., Wagner, E.F., 1997. PARP is important for genomic stability but dispensable in apoptosis. Genes Dev. 11, 2347–2358.

Wang, X., Ohnishi, K., Takahashi, A., Ohnishi, T., 1998. Poly(ADP–ribosyl)ation is required for p53-dependent signal transduction induced by radiation. Oncogene 17, 2819–2825.

Wedge, S.R., Porteous, J.K., Newlands, E.S., 1996. 3-Aminobenzamide and/or O^6-benzylguanine evaluated as an adjunct to temozolomide or BCNU treatment in cell lines of variable mismatch repair status and O^6-alkylguanine–DNA alkyltransferase activity. Br. J. Cancer 74, 1030–1036.

Weller, M., Rieger, J., Grimmel, C., Van Meir, E.G., De Tribolet, N., Krajewski, S., Reed, J.C., von Deimling, A., Dichgans, J., 1998. Predicting chemoresistence in human malignant glioma cells: the role of molecular genetic analyses. Int. J. Cancer 79, 640–644.

Whitacre, C.M., Hashimoto, H., Tsai, M.L., Chatterjee, S., Berger, S.J., Berger, N.A., 1995. Involvement of NAD-poly(ADP–ribose) metabolism in p53 regulation and its consequences. Cancer Res. 55, 3697–3701.

Winter, S., Weller, M., 1998. Potentiation of CD95L-induced apoptosis of human malignant glioma cells by topotecan involves inhibition of RNA synthesis but not changes in CD95 or CD95L expression. J. Pharmacol. Exp. Ther. 286, 1374–1382.

Wright, S.C., Wie, Q.S., Kinder, D.H., Larrick, J.W., 1996. Biochemical pathways of apoptosis: nicotinamide adenine dinucleotide-deficient cells are resistant to tumor necrosis factor or ultraviolet light activation of the 24-kD apoptotic protease and DNA fragmentation. J. Exp. Med. 183, 463–471.

Zhang, J., Dawson, V.L., Dawson, T.M., Snyder, S.H., 1994. Nitric oxide activation of poly(ADP–ribose) synthetase in neurotoxicity. Science 263, 687–689.