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Gemcitabine cytotoxicity of human malignant glioma cells: modulation by antioxidants, BCL-2 and dexamethasone

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Abstract

Gemcitabine is a novel antimetabolite drug that acts by multiple mechanisms, including inhibition of ribonucleoside diphosphate reductase, of dCMP deaminase and of dCTP incorporation into DNA and RNA. Here, we report that gemcitabine induces cytotoxic and clonogenic death of 12 human malignant glioma cell lines at clinically relevant concentrations around 1 μM. Gemcitabine is thus approximately 100-fold more active than the congener drug, cytarabine. Gemcitabine cytotoxicity of glioma cells does not require wild-type p53 activity: (i) there was no difference in the susceptibility to gemcitabine between cell lines with wild-type p53 and cell lines with mutant or deleted p53; (ii) ectopic expression of a temperature-sensitive p53 protein either at wild-type (32.5°C) or at mutant (38.5°C) conformation had no significant influence on gemcitabine-induced cell death. Gemcitabine cytotoxicity was unaffected by the antioxidants, *N*-acetylcysteine and phenyl-*N*-*tert*-butyl-α-phenylnitrone. There was no correlation between the susceptibility to gemcitabine and the endogenous expression of the B cell lymphoma-2 (BCL-2)-family proteins BCL-2, BCL-XL, myeloid cell leukemia-1 (MCL-1), BCL-2-associated X protein (BAX), BCL-2 homologous antagonist/killer (BAK) and BCL-XS. Ectopic expression of BCL-2 moderately attenuated gemcitabine-induced cell death. Similarly, preexposure to the synthetic steroid, dexamethasone, which is commonly used to control cerebral edema in brain tumor patients, reduced gemcitabine cytotoxicity. We conclude that the clinical evaluation of gemcitabine for the adjuvant chemotherapy of malignant glioma is warranted.

Keywords: Glioma, malignant; Gemcitabine; BCL-2 (B cell lymphoma-2); Chemotherapy; Dexamethasone

1. Introduction

2′,2′,-Difluoro-deoxycytidine (gemcitabine) is a nucleoside analogue with significant activity against solid tumor cell lines in vitro and against solid tumors of pancreas and lung in vivo (Plunkett et al., 1995; Guchelaar et al., 1996; Arning and Blatter, 1997; Noble and Goa, 1997). Myelosuppression is the dose-limiting toxicity. After gemcitabine has crossed the plasma membrane, it is phosphorylated by deoxycytidine kinase-dependent phosphorylation, resulting in the formation of the monophosphate, 2′,2′-difluoro-deoxycytidine monophosphate (dFdCMP). Loss of deoxycytidine kinase has been associated with resistance to gemcitabine (Ruiz van Haperen et al., 1994), an evidence for the importance of deoxycytidine kinase for gemcitabine cytotoxicity. Further phosphorylation of dFdCMP results in the formation of the respective di- and triphosphates, dFdCDP and dFdCTP, which are thought to be the active metabolites of gemcitabine. dFdCDP and, to a lesser extent, dFdCTP, inhibit ribonucleoside diphosphate reductase (Heinemann et al., 1990), resulting in reduced production of deoxynucleotides. dFdCTP is incorporated into DNA and inhibits DNA synthesis. Since one or more nucleotides may be incorporated into DNA after a gemcitabine derivative has been incorporated, chain termination has been referred to as masked and may not be amenable to DNA repair (Gandhi et al., 1996). These pathways of action of gemcitabine have been associated with metabolic self-potentiation: reduction of dCTP formation by inhibition of ribonucleotide reductase, and, at high concentration, of CTP synthetase (Heinemann et al., 1995) lead to increased incorporation of dFdCTP into DNA since dFdCTP competes with dCTP for incorporation into DNA. Further, since deoxycytidine kinase is inhibited by deoxycytidine and dCTP, reduced levels of dCTP and deoxycytidine may

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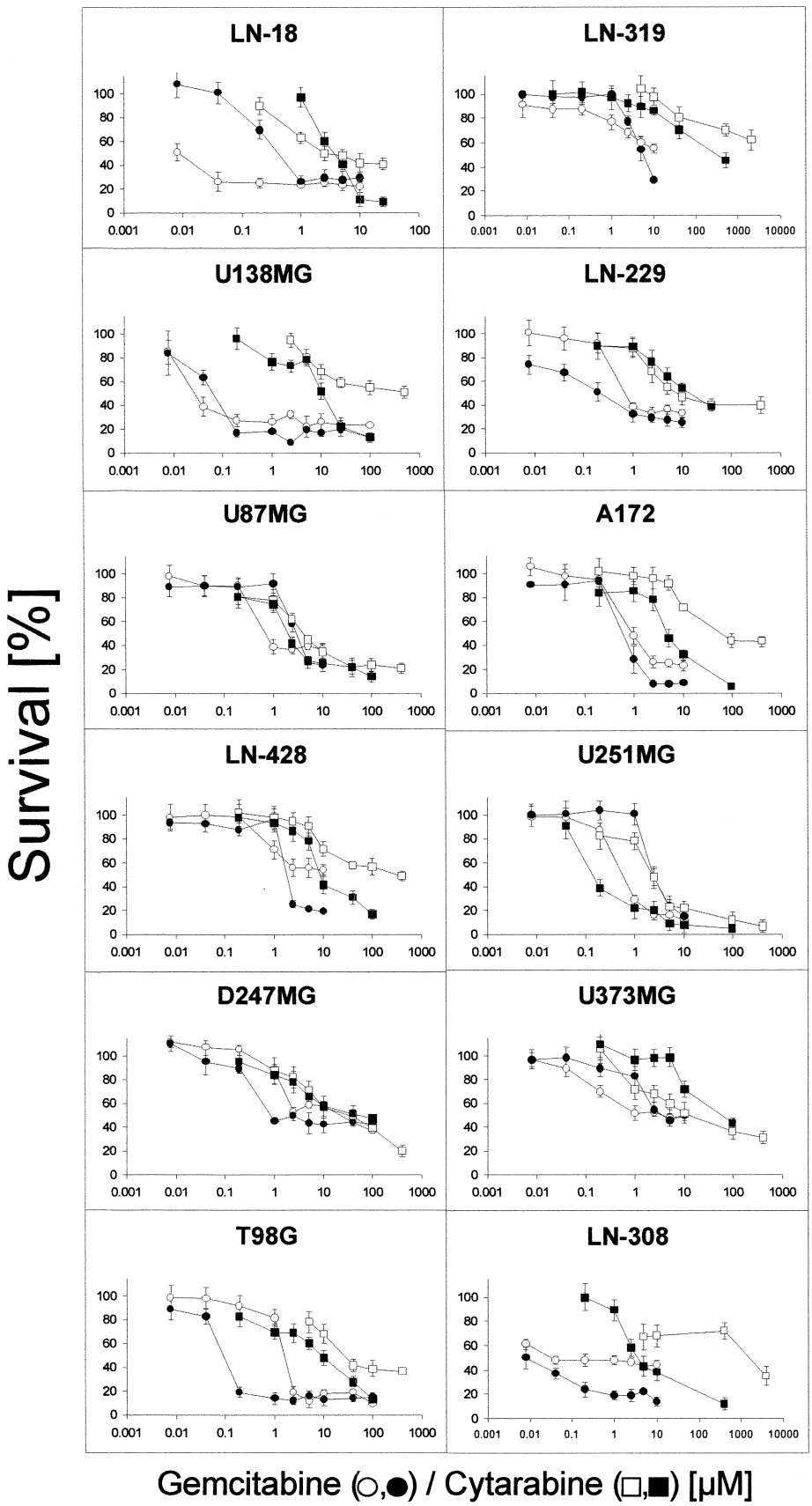
LN-18
LN-319
U138MG
LN-229
U87MG
A172
LN-428
U251MG
D247MG
U373MG
T98G
LN-308
Survival [%]
Gemcitabine (○,●) / Cytarabine (□,■) [μM]

Table 1
Drug-induced cytotoxic cell death: a comparison of EC_{50} values

	GEM (μM)	Ara-C (μM)	BCNU (μM)	VCR (μM)	VM26 (μM)	DXR (μM)	CPT (μM)	β-Lap (μM)
LN-18	0.01	1	300	0.08	1	0.45	0.04	0.4
U138MG	0.02	500	200	0.2	4	0.80	0.3	1.5
U87MG	0.8	3	60	0.2	2	0.35	0.12	0.5
LN-428	10	400	300	0.02	0.70	0.25	0.1	1.5
D247MG	12	20	320	0.015	10	0.08	0.1	0.5
T98G	1.5	25	500	0.08	1.20	1.50	0.08	0.8
LN-319	25	2000	900	0.5	1.50	1	3.5	1.5
LN-229	0.8	15	130	0.06	1.10	0.09	0.1	0.4
A172	0.9	30	475	0.16	2.20	0.20	0.07	1.6
U251MG	0.8	1.50	25	0.03	0.20	0.15	0.01	1.5
U373MG	1	12	450	0.13	0.70	0.40	0.1	1.2
LN-308	0.02	1500	30	0.6	8	1.50	1.2	0.6

The cells were treated for 72 h with gemcitabine (GEM), cytarabine (Ara-C), BCNU, vincristine (VCR), teniposide (VM26), doxorubicine (DXR), camptothecin (CPT) or β-lapachone (β-Lap).
EC_{50} values were determined as described in Section 2.
Data are mean values ($n = 4$).
Note that the data for all drugs except gemcitabine have been previously reported (Weller et al., 1998) and are shown here for comparison only.

result in increased phosphorylation of gemcitabine. In addition, inhibition of dCMP deaminase, the rate-limiting enzyme for the elimination of gemcitabine nucleotides from the cell, by reducing dCTP, a cofactor of dCMP deaminase, and by direct inhibition by dFdCTP, might lead to the prolonged retention of gemcitabine nucleotides, compared with cytarabine. Finally, reduced cellular deoxynucleotides, depletion of cellular dATP pools (Shewach et al., 1994; Shewach and Lawrence, 1995), and impaired DNA repair by masked chain termination are linked to gemcitabine-mediated radiosensitization. As a final common pathway, gemcitabine has been shown to induce apoptosis in various tumor cell lines (Bouffard and Momparler, 1995; Gruber et al., 1996).

Here, we characterize the effects of gemcitabine on human malignant glioma cell lines in vitro. Since p53 status and expression of BCL-2 family proteins are thought to determine cancer cell response to proapoptotic stress (Adams and Cory, 1998, Weller, 1998), we investigated the role of these proteins in gemcitabine-induced glioma cell death. Further, we examined whether the synthetic steroid, dexamethasone, modified gemcitabine-induced cell death. This is because dexamethasone has been shown to reduce cytotoxic drug-induced cell death in glioma cells (Weller et al., 1997a; Naumann et al., 1998) but is still commonly used for the control of cerebral edema in human brain tumor patients.

2. Materials and methods

2.1. Reagents and cell lines

2′,2′,-Difluoro-deoxycytidine (gemcitabine) was kindly provided by Lilly (Homburg, Germany). *N*-acetylcysteine and phenyl-*N*-*tert*-butyl-α-phenylnitrone were purchased from Sigma (St. Louis, MO). A172, LN-229, LN-18, LN-308, LN-319, LN-428, U87MG, U138MG, U251MG, U373MG and D247MG human malignant glioma cells were kindly provided by Dr. N. de Tribolet (Lausanne, Switzerland). The glioma cells were cultured in Dulbecco's modified eagle medium (DMEM) containing 10% fetal calf serum, 2 mM glutamine, and 1% penicillin/streptomycin.

2.2. Transfections

Human glioma cell lines engineered to express the murine B cell lymphoma (BCL)-2 gene or murine p53val^{135} and the respective neo or hygro control cell lines have been described (Weller et al., 1995; Trepel et al., 1998). Murine p53val^{135} is a temperature-sensitive p53 mutant which assumes mutant conformation at 38.5°C but acquires wild-type conformation and functional properties at 32.5°C. Transfections were carried out using electroporation (Bio-rad Gene Pulser, 250 V, 950 μF). Selection was done with

Fig. 1. Gemcitabine induces cytotoxic and clonogenic cell death in human malignant glioma cell lines. The glioma cell lines were treated with gemcitabine (circles) or cytarabine (squares), either for 72 h and survival determined immediately thereafter (open symbols), or pulse-treated for 24 h and allowed to recover for 5–10 generation times (filled symbols) as described in Section 2. Survival was assessed by crystal violet staining. Data are expressed as mean percentages of survival and S.E.M. ($n = 3$).

Table 2
Drug-induced clonogenic cell death: a comparison of EC_{50} values

	GEM (μM)	Ara-C (μM)	BCNU (μM)	VCR (μM)	VM26 (μM)	DXR (μM)	CPT (μM)	β-Lap (μM)
LN-18	0.1	3	210	1.5	0.8	0.4	0.3	0.3
U138MG	0.05	13	35	0.5	1	0.25	0.3	1.7
U87MG	3	1.5	100	0.07	0.5	0.2	0.3	1
LN-428	2	8	75	0.2	0.4	0.1	0.1	0.5
D247MG	1	30	150	0.2	2.5	0.5	0.9	0.4
T98G	0.1	9	300	0.1	0.7	0.2	0.025	0.2
LN-319	5.5	200	120	0.5	0.8	0.4	0.75	1.4
LN-229	0.5	16	50	0.05	1	0.2	0.07	0.2
A172	0.8	2.5	20	0.15	0.3	0.2	0.07	0.4
U251MG	2.5	0.2	25	0.05	0.4	0.1	0.1	0.4
U373MG	3.5	50	60	0.7	0.7	0.25	0.4	1.4
LN-308	0.008	3	40	1	2.6	0.7	0.4	0.4

The cells were pulse-treated for 24 h with gemcitabine (GEM), cytarabine (Ara-C), BCNU, vincristine (VCR), teniposide (VM26), doxorubicine (DXR), camptothecin (CPT) or β-lapachone (β-Lap), allowed to recover for 5–10 generation times.
The EC_{50} values were determined as described in Section 2.
Data are mean values ($n = 4$).
Note that the data for all drugs except gemcitabine have been previously reported (Weller et al., 1998) and are shown here for comparison only.

G418 (neo, 0.5 mg/ml) or hygromycin B (hygro, 200 μg/ml). Expression of the transgenes was ascertained by immunoblot analysis for BCL-2 and p53 as described (Weller et al., 1995; Trepel et al., 1998) (data not shown).

2.3. Viability assays

For acute cytotoxicity assays, the cells were seeded at 1×10^4 cells per well in 96-well plates, adhered for 24 h, and exposed to the drugs for 72 h. Most cell lines double twice, except LN-319, LN-308 and LN-428 cells which have doubling times of 50–60 h, and the cells grow exponentially within the time frame of this experiment (Weller et al., 1998). Survival was assessed by crystal violet staining. For clonogenic survival assays, the cells were seeded at 1×10^3 cells per well, adhered for 24 h, pulse-treated for 24 h with the drugs, and maintained drug-free for 5–10 generation times in complete medium. Proliferation was assessed by crystal violet staining. Briefly, the cell culture medium was removed and surviving cells stained with 0.5% crystal violet in 20% methanol for 20 min at room temperature. The plates were washed extensively under running tap water, air-dried and optical density values read in an enzyme-linked immunosorbent assay (ELISA) reader at 550 nm wave length. For the determination of EC_{50} values, the drug effects were monitored in serial dilutions over a broad range of concentrations.

2.4. Statistical analysis

Correlations between EC_{50} values were computed by Pearson product-moment correlational analysis. For comparisons of two groups, a *t*-test for independent samples was used. Results were considered significant at a P level of $P < 0.05$.

3. Results

3.1. Characterization of gemcitabine cytotoxicity of human malignant glioma cell lines

The cytotoxic and anticlonogenic effects of gemcitabine were studied in 12 human malignant glioma cell lines (Fig. 1). The EC_{50} values for the induction of cytotoxic cell death (Table 1) ranged between 0.01 μM (LN-18) and 25 μM (LN-319). Thus, the EC_{50} values were much lower than those of the related drug, cytarabine, which are between 1 and 2000 μM (Table 1, Weller et al., 1998).

Table 3
Gemcitabine cytotoxicity of glioma cells: *r*-values for correlations of EC_{50} values for other drugs

	Gemcitabine-induced cytotoxic cell death	Gemcitabine-induced clonogenic cell death
Ara-C	0.61*	0.75*
BCNU	0.70*	−0.16
VCR	0.28	−0.17
VM26	0.11	−0.34
DXR	0.05	−0.18
CPT	0.77*	0.38
β-Lap	0.27	0.53

The EC_{50} values for gemcitabine were correlated with the EC_{50} values for cytarabine (Ara-C), BCNU, vincristine (VCR), teniposide (VM26), doxorubicine (DXR), camptothecin (CPT) and β-lapachone (β-Lap) in cytotoxic (left panel) and clonogenic (right panel) cell death assays.
Expressed are *r*-values which were determined by Pearson product-moment correlation as described in Section 2 (*, significant correlation, $P < 0.05$).

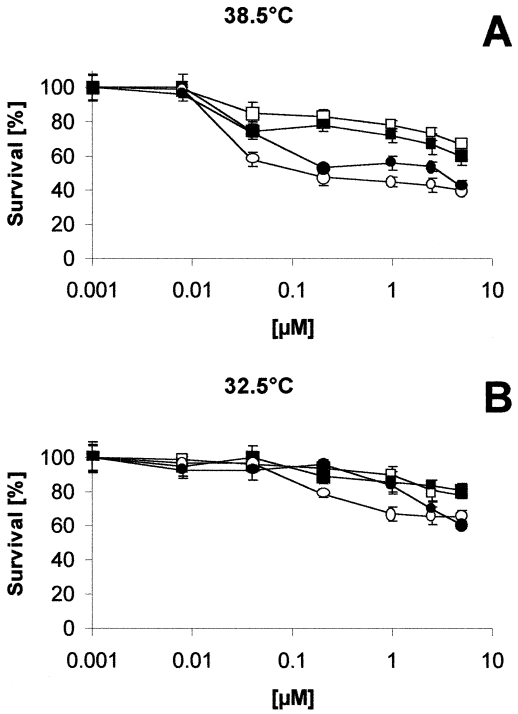

Fig. 2. Ectopic expression of mutant or wild-type p53 has little impact on gemcitabine-induced cell death in human malignant glioma cells. (A, B) p53val^{135}-transfected (filled symbols) or hygro control sublines (open symbols) of LN-18 (circles) or LN-229 (squares) cells were exposed to gemcitabine for 72 h at 38.5°C (mutant p53) (A) or, after 4 h adaptation to 32.5°C, for 72 h at 32.5°C (wild-type p53) (B) Survival was measured by crystal violet assays. Data are expressed as mean percentages of survival and S.E.M. ($n = 3$).

The fact that even high concentrations of gemcitabine resulted in the survival of up to 40% of the cells in some cell lines indicates cytostatic rather than cytotoxic actions of gemcitabine in these assays.

The EC_{50} values for the induction of clonogenic cell death by gemcitabine (Table 2) were somewhat lower than the values obtained in the cytotoxic cell death assays and ranged between 0.008 μM (LN-308) and 5.5 μM (LN-319). Correlation analysis revealed a significant correlation between the EC_{50} values for cytotoxic and clonogenic cell death induced by gemcitabine ($r = 0.65$, $P < 0.05$).

The EC_{50} values for gemcitabine cytotoxicity were also compared with the EC_{50} values determined previously for 1,3-bis(2-chloroethyl)1-nitrosurea (BCNU), vincristine, cytarabine, teniposide (VM26), doxorubicine, camptothecin and β-lapachone (Table 3, Weller et al., 1998). There were positive correlations ($P < 0.05$) between the EC_{50} values for cytotoxic cell death induced by gemcitabine and the EC_{50} values of cytotoxic cell death induced by cytarabine, camptothecin and BCNU. The EC_{50} values for clonogenic cell death induced by gemcitabine correlated significantly with the EC_{50} values of clonogenic cell death induced by cytarabine. There was no correlation between the doubling time of the cell lines (Weller et al., 1998) and EC_{50} values for gemcitabine-induced cytotoxic or clonogenic cell death.

3.2. p53 status has no significant effect on gemcitabine cytotoxicity of malignant glioma cells

The genetic and functional status of the p53 tumor suppressor gene is thought to be an important predictor of response to chemotherapy (Weller, 1998). The p53 status of the glioma cell lines examined here has been determined previously: U87MG, D247MG and A172 are wild-type genetically, and LN-229 is heterozygous genetically but exhibits p53 transcriptional activity (Van Meir et al., 1994) and accumulates p53 in response to genotoxic stress (Weller et al., 1997b), both consistent with retention of wild-type function. The other eight cell lines lack wild-type p53 function. When the EC_{50} values of these four cell lines were compared with the EC_{50} values of the other eight cell lines, no significant difference became apparent ($P > 0.05$), indicating that cell death induced by gemcitabine is not dependent on the presence of wild-type p53 function.

To investigate the effects on gemcitabine cytotoxicity of altering the p53 status of malignant glioma cells, we took advantage of glioma cell sublines expressing the temperature-sensitive murine p53val^{135} mutant (Trepel et al., 1998). Ectopic expression of mutant p53 (38.5°C) had no significant effect on gemcitabine cytotoxicity in LN-18 or LN-229

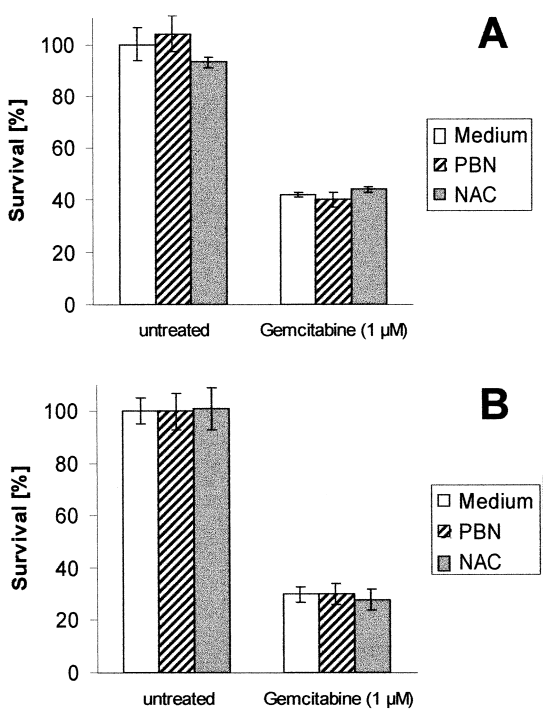

Fig. 3. The antioxidants, *N*-acetylcysteine and phenyl-*N*-*tert*-butyl-α-phenylnitrone, do not influence gemcitabine-induced cell death in human malignant glioma cells. (A, B) LN-229 (A) or LN-18 (B) cells were exposed to gemcitabine in the absence or presence of *N*-acetylcysteine (100 μM) (NAC) or phenyl-*N*-*tert*-butyl-α-phenylnitrone (150 μM) (PBN) for 72 h. Survival was measured by crystal violet assays. Data are expressed as mean percentages of survival and S.E.M. ($n = 3$) ($P > 0.05$).

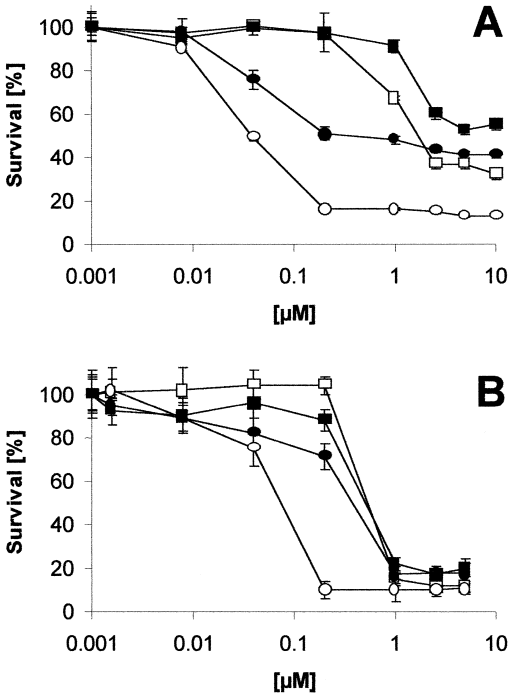

Fig. 4. Ectopic expression of BCL-2 inhibits gemcitabine-induced cell death. (A, B) Bcl-2-transfected (filled symbols) or neo control (open symbols) cells (LN-18, circles; LN-229, squares) were exposed to gemcitabine for 72 h (A) or in 24 h pulse assays (B) as in Fig. 1. (B) Survival or growth in A and B were measured by crystal violet assays. Data are expressed as mean percentages of growth/survival and S.E.M. ($n = 3$). One representative experiment of three independent experiments with similar results is shown. The EC_{50} values are compared in Table 4.

cells (Fig. 2A). Ectopic expression of wild-type p53 induced moderate resistance to gemcitabine in LN-18 cells at around 1 μM gemcitabine whereas no effect was observed in LN-229 cells (Fig. 2B). Gemcitabine cytotoxicity was slightly reduced by forced expression of p53 in T98G and LN-308 cells at both temperatures (data not shown) but these effects did not reach statistical significance at the level of EC_{50} values. These data are in line with the observation that the endogenous p53 status is not predictive of sensitivity to gemcitabine.

3.3. Antioxidants do not modulate gemcitabine-induced cell death

Free radical formation is an important step in many instances of apoptosis and may mediate p53-induced apoptosis (Polyak et al., 1997). Consistent with this concept, antioxidants often protect cells from apoptosis. We find that two antioxidants that are active in preventing neuronal apoptosis (Schulz et al., 1997), *N*-acetylcysteine and the free radical spin trap, phenyl-*N*-*tert*-butyl-α-phenylnitrone, do not modulate gemcitabine cytotoxicity (Fig. 3).

3.4. BCL-2 inhibits gemcitabine cytotoxicity of human malignant glioma cells

The proto-oncogene product, BCL-2, is a potent inhibitor of apoptosis in many paradigms of apoptosis, including chemotherapy-induced cell death (Weller et al., 1995). We find that the endogenous expression levels of the BCL-2 family proteins, BCL-2, BCL-2-associated X protein (BAX), BCL-X_L, BCL-2 homologous antagonist/killer (BAK), BCL-X_s and myeloid cell leukemia-1 (MCL-1) (Weller et al., 1998) did not predict sensitivity or resistance to cytotoxic or clonogenic cell death induced by gemcitabine. However, consistent with the antiapoptotic action of BCL-2 in malignant glioma cells exposed to cytotoxic cytokines (Weller et al., 1995), cytotoxic cell death of LN-18 and LN-229 cells was inhibited by ectopic expression of a murine BCL-2 transgene (Fig. 4): there was an EC_{50} shift from 0.9 to 4.4 μM in LN-229 cells and from 0.02 to 0.1 μM in LN-18 cells (Table 4). In clonogenic cell death assays, the protective effect of BCL-2 became apparent in LN-18 cells only. There was an EC_{50} shift from 0.08 to 0.4 μM. No such effect was seen for LN-229 cells.

3.5. Gemcitabine cytotoxicity of malignant glioma: modulation by dexamethasone

Human malignant glioma patients are often treated with steroids for the control of cerebral edema. However,

Table 4
Gemcitabine cytotoxicity of malignant glioma cells: modulation of EC_{50} values by BCL-2 and dexamethasone

	Cytotoxic cell death assays (μM)		Clonogenic cell death assays (μM)		Cytotoxic cell death assays (μM)		Clonogenic cell death assays (μM)	
	Neo	BCL-2	Neo	BCL-2	Untr.	DEX	Untr.	DEX
LN-229	0.9	4.4∗	0.4	0.4	0.7	1.4∗	0.5	0.5
LN-18	0.02	0.1∗	0.08	0.4∗	0.01	0.02∗	0.1	0.11

Left: EC_{50} values of BCL-2 transfected (BCL-2) or mock-transfected (neo) LN-229 or LN-18 cells were determined in cytotoxic and clonogenic cell death assays as described in Section 2 from three independent experiments (∗ $P < 0.05$, *t*-test).
Right panel: LN-18 and LN-229 cells were either treated with gemcitabine alone (untr.) or preexposed to dexamethasone (DEX) (100 nM) for 24 h and then cotreated with gemcitabine and DEX.
The EC_{50} values for cytotoxic and clonogenic cell death assays were determined as described in Section 2 from three independent experiments (∗ $P < 0.05$, unpaired *t*-test).

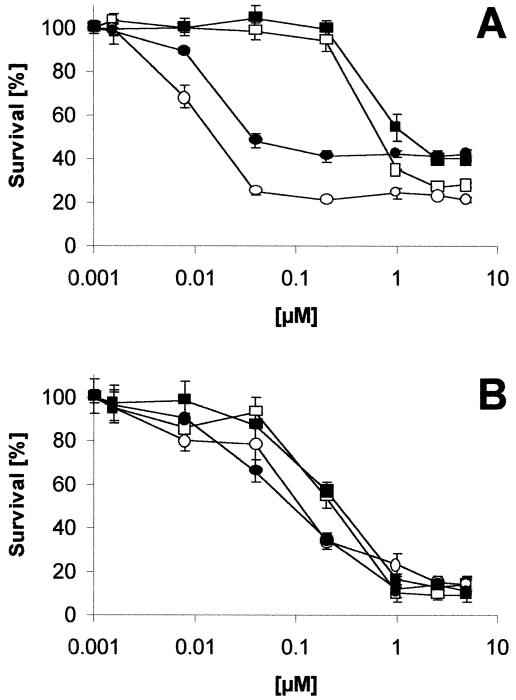

Fig. 5. Modulation of gemcitabine induced cell death by dexamethasone. (A, B) LN-18 (circles) or LN-229 (squares) cells were exposed to gemcitabine alone (open symbols) or were pretreated with dexamethasone (100 nM) (filled symbols) for 24 h and then cotreated with dexamethasone and gemcitabine in acute cytotoxicity (A) or clonogenic cell death (B) assays. Survival or growth in (A) and (B) were measured by crystal violet assays. Data are expressed as mean percentages of growth/survival and S.E.M. ($n = 3$). One representative experiment of three independent experiments with similar results is shown. The EC_{50} values are compared in Table 4.

steroids may interfere with the efficacy of chemotherapy in these neoplasms (Weller et al., 1997a; Naumann et al., 1998). Assessing a modulation of drug cytotoxicity by steroids is therefore of significant clinical relevance. The effects of gemcitabine were indeed moderately inhibited by clinically relevant concentrations of dexamethasone when cytotoxic cell death was assayed (Fig. 5A). There was a minor EC_{50} shift from 0.7 to 1.4 μM for LN-229 cells and from 0.01 to 0.02 μM for LN-18 cells (Table 4). In contrast, no protective effect of dexamethasone was detected in clonogenic cell death assays (Fig. 5B).

4. Discussion

Since cytoreductive surgery and involved-field radiotherapy fail to prolong the median survival for human malignant glioma patients beyond 1 year, the identification of novel cytotoxic agents with activity against malignant glioma is of utmost clinical importance. Gemcitabine is a novel nucleoside analogue with significant preclinical and clinical activity against some solid tumors (Plunkett et al., 1995; Guchelaar et al., 1996; Arning and Blatter, 1997; Noble and Goa, 1997).

In this first study of the effects of gemcitabine on cultured human malignant glioma cells, we report that human glioma cell lines are susceptible to the cytotoxic and anticlonogenic actions of gemcitabine at clinically relevant concentrations (Fig. 1, Tables 1 and 2). Not surprisingly, there was a significant correlation between the cellular sensitivity to gemcitabine and the sensitivity to the related agent, cytarabine, consistent with previous results (Csoka et al., 1995). However, gemcitabine was approximately a 100-fold more potent than cytarabine, similar to previous reports on leukemia cell sensitivity to these agents (Bouffard et al., 1991). The concentrations required for the induction of cytotoxic cell death in gemcitabine-sensitive glioma cell lines (LN-18, U138MG, LN-308) were comparable with those reported for leukemia cell lines (50 nM) (Bouffard and Momparler, 1995), prostate carcinoma cells (30 nM) (Cronauer et al., 1996) and some myeloma cell lines (10 nM) (Gruber et al., 1996). Interestingly, there was cross-sensitivity of the glioma cell lines to gemcitabine and the topoisomerase I inhibitor, camptothecin, an observation that is difficult to explain at present.

The cytotoxic and anticlonogenic effects of gemcitabine did not depend on the endogenous p53 status of the cell lines. In line with this observation, ectopic expression of p53 either in wild-type or mutant conformation had no major effect on cell death induced by gemcitabine (Fig. 2). Gemcitabine cytotoxicity was unaffected by antioxidants, suggesting that free radical formation does not mediate gemcitabine cytotoxicity of human malignant glioma cells (Fig. 3). No correlation between the expression of BCL-2 family proteins and susceptibility to gemcitabine became apparent, indicating that other mechanisms might be responsible for the differential resistance of glioma cells to gemcitabine. However, ectopic expression of BCL-2 attenuated gemcitabine-induced cell death (Fig. 4, Table 4). The fact that the protective effect of BCL-2 was more obvious in cytotoxic cell death assays than in clonogenic cell death assays suggests that BCL-2 inhibits acute cytotoxicity of gemcitabine rather than gemcitabine-induced growth inhibition, consistent with the known antiapoptotic properties of BCL-2.

Based on cell culture studies and clinical considerations, we have previously called for a judicious use of steroid medications for edema control in human glioma patients undergoing chemotherapy (Weller et al., 1997a). Pre-exposure to dexamethasone moderately reduced gemcitabine-induced cell death of the glioma cells, in line with previous results with other cytotoxic drugs even though the protection afforded by dexamethasone was moderate at best (Fig. 5, Table 4). Steroid withdrawal would thus also be predicted to enhance the effects of gemcitabine chemotherapy of human malignant glioma in vivo, specifically

when considering that dexamethasone reduces tumor perfusion and stabilizes blood–tumoral barriers as well.

The penetration of gemcitabine through the intact blood brain barrier is probably low. After a single dose of 10 mg/kg in the rat, cerebral tissue levels peaked at 2 h at 1.1 μg/ml (3.7 nM) and returned to 0.16 μg/ml (0.53 nM) at 24 h and 0.02 μg/ml (0.066 nM) at 5 days (Esumi et al., 1994). However, human malignant gliomas are not shielded from a true blood brain barrier, and poor tumor perfusion may be more relevant clinically than protection from blood tumoral barriers. Maximal plasma levels are in the range of 30–40 μM but the plasma half-life is probably below 30 min (Guchelaar et al., 1996; Arning and Blatter, 1997). Taken together, these cell culture data have encouraged us to initiate a phase I/II chemotherapy trial of gemcitabine for malignant glioma at our institution.

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