ELSEVIER

Biochemical Pharmacology 63 (2002) 881–887

Biochemical Pharmacology

Fas/CD95-mediated apoptosis in human glioblastoma cells: a target for sensitisation to topoisomerase I inhibitors

Emilio Ciusani[a], Paola Perego[b], Nives Carenini[b], Elisabetta Corna[b], Federica Facchinetti[b], Amerigo Boiardi[a], Andrea Salmaggi[a], Franco Zunino[b,*]

[a]*Istituto Neurologico C. Besta, 20133 Milan, Italy*
[b]*Istituto Nazionale per lo Studio e la Cura dei Tumori, 20133 Milan, Italy*

Received 16 March 2001; accepted 25 September 2001

Abstract

The expression of the death receptor Fas/CD95 is cell type-specific and can be modulated by different cytotoxic treatments. In spite of a frequent expression of Fas/CD95 in high-grade gliomas, these tumours are typically refractory to conventional therapy. Using a human glioblastoma cell line (GBM), we explored the possibility of modulating susceptibility to Fas/CD95-mediated apoptosis following cytotoxic treatment. GBM cells were sensitive to the antiproliferative effects of topoisomerase I inhibitors (topotecan and a novel lipophilic analog CPT83) and taxol, less sensitive to cisplatin and, in any case, rather resistant to drug-induced apoptosis. This pattern of cellular response was consistent with *p53* mutation. GBM cells expressed low levels of Fas/CD95, which was associated with low susceptibility to antibody-stimulated Fas/CD95-mediated apoptosis. A significant up-regulation of Fas/CD95 expression was detected after exposure to topotecan and CPT83, whereas cisplatin induced a low increase and taxol did not modify Fas/CD95 expression. In addition, after treatment with topoisomerase I inhibitors, the up-regulation of Fas/CD95 resulted in an increased susceptibility of GBM cells to antibody-stimulated Fas/CD95-mediated apoptosis, while no synergistic effects were detected after treatment with cisplatin or taxol. Our data suggest that Fas/CD95 up-regulation can be a common response to DNA damage, whereas sensitisation to Fas/CD95-mediated apoptosis appears to be dependent on the type of DNA damage and on the pathway of cellular response. The observed effects might have important therapeutic implications for the design of novel therapeutic strategies in the treatment of malignant gliomas.

Keywords: Fas/CD95; Human glioblastoma; Topoisomerase I inhibitors; Apoptosis; DNA damaging agents; Taxol

1. Introduction

Malignant gliomas are aggressive tumours which are often detected already at advanced stage. The post-surgical treatment of malignant glioma usually involves radiotherapy, but new strategies are required to increase the therapeutic outcome [1]. Cellular studies on *ex vivo* isolated cell lines have documented that the intrinsic resistance of malignant glioma could be related to the presence of multiple alterations including those affecting genes involved in triggering the apoptotic response (i.e. *p53*) [2,3].

Fas/CD95/Apo1 is a transmembrane receptor known to trigger programmed cell death in several cell types [4,5]. The activation of Fas/CD95 by the binding of its natural ligand (Fas/CD95-ligand) or by specific antibodies, transduces an apoptotic signal that activates members of a class of cysteine proteases starting a cascade of events leading to DNA fragmentation and cell death [4]. Up-regulation of Fas/CD95 has been shown to be related to an increased susceptibility to Fas/CD95-dependent apoptosis in leukemia, melanoma and in colon adenocarcinoma cells [6–8]. Similarly, up-regulation of Fas/CD95 on glioma cells could render them more prone to apoptosis induced by neighbouring Fas/CD95-ligand positive cells: these might include microglia or infiltrating blood-derived monocytes [9].

Since Fas/CD95 is expressed in most of high grade gliomas [10], the aim of this study was to explore the possibility of modulating susceptibility to Fas/CD95-induced apoptosis by antitumour drugs of different classes. Our results indicate that sensitisation to Fas/CD95-mediated apoptosis can be obtained following up-regulation of Fas/CD95 by selected DNA damaging agents.

* Corresponding author. Tel.: +39-2-23902267; fax: +39-2-23902692.
E-mail address: zunino@istitutotumori.mi.it (F. Zunino).

0006-2952/02/$ – see front matter
PII: S0006-2952(01)00837-1

2. Materials and methods

2.1. Drugs

The drugs used in this study were prepared as follows: topotecan was primarily dissolved in water, CPT83 in dimethyl sulfoxide, and taxol in ethanol. For cisplatin (Platinex, Bristol Myers Squibb) and mitoxantrone (Novantrone, Wyeth Lederle), the clinical preparations were used. All working solutions were in saline with the exception of CPT83 which was in water.

2.2. Cell line and evaluation of cell sensitivity to drugs

GBM [11], a human glioblastoma cell line was cultured in RPMI-1640 medium supplemented with 10% foetal calf serum. The cell sensitivity to antitumour agents was evaluated by using the growth-inhibition assay. Cells were seeded in duplicate into 6-well plates (3.5 cm diameter) and exposed to drug 24 hr later. After 24 hr drug incubation, the medium was replaced with fresh medium. Cells were trypsinised and harvested 48 hr later for counting with a cell counter. IC_{50} is defined as the drug concentration producing 50% decrease of cell growth.

2.3. Apoptosis measurement

The percentage of apoptotic cells was evaluated by flow cytometry, as reported by Spinozzi *et al.* [12]. Since apoptotic cells tend to detach from the culture plate and to float in the medium, this analysis was performed on non-adherent and adherent cells together. Twenty-four hours after drug exposure, medium was replaced with fresh medium and cells were harvested at different times. In some experiments, an anti-Fas/CD95 antibody (Kamiya Corporation) capable of inducing Fas/CD95-dependent apoptosis was added to the medium (1 mg/mL) for 24, 48 or 72 hr before harvesting cells. Floating cells were collected from the medium by centrifugation, while adherent cells were trypsinised. Total cells were either processed for Annexin V-binding following manufacturer's instructions (Bender MedSystem) or for propidium iodide staining. In the latter case, cells were resuspended in medium, centrifuged 5 min at 200 *g*, fixed in 80% ethanol and kept at 4° for 2 hr. Cells were then washed and resuspended in phosphate-buffered saline containing 25 μg/mL propidium iodide and analysed after at least 30 min. Nuclear fluorescence was evaluated by flow cytometry using a FACStar Plus and data analysed with a specific software (Cell Quest, Becton-Dickinson).

2.4. p53 gene studies

The *p53* gene analysis was performed by single strand conformation polymorphism analysis followed by sequencing of the shifted bands as previously described [13].

2.5. Fas/CD95 expression

For the analysis of Fas/CD95, GBM cells were incubated for 24 hr in drug-containing medium. Cells were then harvested and incubated for 30 min at 4° with 10 μL of phycoerythrin-conjugated anti-Fas/CD95 monoclonal antibody (Becton-Dickinson) washed twice with phosphate-buffered saline and fixed in 2% paraformaldehyde. Antibody binding was detected by flow cytometry (FACStar Plus, Becton-Dickinson).

2.6. PCR analysis for Fas/CD95 transcripts

Total RNA from GBM cells was isolated using a commercially available kit (Talent, Srl.). RNA was also extracted from peripheral lymphocytes used as positive control. One microgram of RNA was reversed-transcribed into cDNA using oligo(dT) primers and Moloney murine leukemia virus reverse transcriptase (Life Technologies). To amplify both normal Fas/CD95 transcript (275 bp) and Fas/CD95ΔTM (234 bp), the MB-24-27 (5′-CTGTTTCAGGATTTAAGGTTGGAGATT-3′) and the MB-59-26 (5′-GACCCAGAATACCAAGTGCAGATGTA-3′) primers were used [14]. PCR conditions were as follows: 94°, 3 min; 62°, 1 min; 72°, 1 min for 1 cycle; 94°, 1 min; 62°, 1 min; 72°, 1 min for the remaining 29 cycles, followed by a 10 min extension at 72°. The amplification products were separated on a 1.5% agarose gel. To amplify both normal (175 bp) and exon 8 deletion (150 bp) Fas/CD95 transcript, lacking the death signalling domain, we used GR19-20 (5′-AACATGCAGAAAGCACAGAA-3′) and GR46-26 (5′-CATTGACACCATTCTTTCGAACAAG-3′) primers [14,15]. PCR conditions were identical to those described above with the exception of annealing that was at 58°. The amplification products were separated on a 2% agarose gel with ethidium bromide and UV-visualised using a VDS Image Master (Amersham Pharmacia Biotech Italia). The intensity of the bands was quantified using a PhosphorImager-dedicated software (Molecular Dynamics, Amersham Pharmacia Biotech).

2.7. Measurement of RNA synthesis

Cells were seeded in 96-well plates (9×10^4 cells/mL) and grown for 48 hr. To determine the effects of the studied drugs on RNA synthesis, cells were pulse-labelled with 10 μCi/mL ^{3}H-uridine (specific activity 39 Ci/mmol) for 4 hr in the presence of cytotoxic concentrations (corresponding to IC_{80}) of cisplatin, topotecan, CPT83 or taxol. Cells were then harvested and spotted (Harvester 96, Tomtec) onto a filter (Printed Filtermat A) counted in a liquid scintillation counter (1205 Beta Plate™).

2.8. Statistical analysis

Statistical analysis was performed by using the Mann–Whitney *U*-test. Level of significance was set at 0.05.

3. Results

3.1. Cell sensitivity to drugs and to apoptosis induction

The sensitivity of GBM cells to antitumour drugs of different classes including topoisomerase I inhibitors (topotecan, CPT83), cisplatin and taxol was evaluated using the growth–inhibition assay after 24 hr drug exposure (Table 1). The pattern of chemosensitivity to cytotoxic agents was somewhat expected on the basis of the mechanism of action. These cells showed a marked sensitivity to camptothecins. The increased activity of the novel analog CPT83 is likely related to its lipophilic nature [16].

Since apoptosis is a critical mode of cell death induced by antitumour agents in sensitive cells, we investigated the capability of the drugs to induce apoptotic cell death (72 hr after drug exposure). GBM cells were rather resistant to drug-induced apoptosis as shown by an analysis of apoptosis levels in propidium iodide-stained cells (Fig. 1). Similar results were obtained using the Annexin V-binding assay. Therefore, further experiments were performed with propidium iodide staining. Our observation suggests that apoptosis did not account for the antiproliferative effects.

Table 1
Pattern of cellular sensitivity of GBM cells to different drugs[a]

	IC_{50}[b]
Topotecan	0.035 ± 0.02
CPT83	0.0047 ± 0.0007
Cisplatin	0.13 ± 0.003
Taxol	0.016 ± 0.007

[a] Cytotoxicity was assessed by growth inhibition assay after 24 hr drug exposure: cells were counted 48 hr after the end of incubation with drug. Values are the mean (±SD) of at least three independent experiments.
[b] IC_{50} (μg/mL), concentration inhibiting 50% of cell growth.

3.2. p53 gene analysis

To analyse the status of the *p53* gene, we performed single strand conformation polymorphism analysis followed by sequencing analysis. The GBM cells exhibited a mutation affecting codon 282 (CGG → TGG, Arg → Trp).

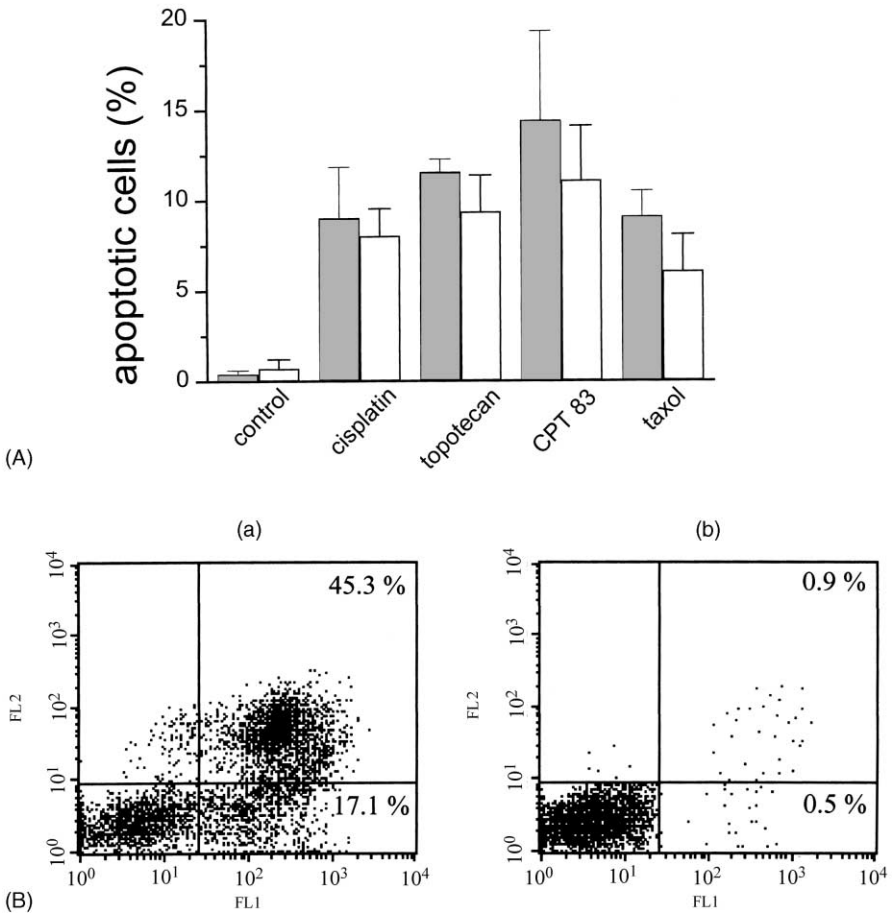

Fig. 1. Susceptibility to drug-induced apoptosis of GBM cells. Cells were exposed to a cytotoxic concentration (corresponding to IC_{80}) of different drugs for 24 hr. (A) Apoptosis was measured 72 hr later through cytofluorimetric analysis of propidium iodide-stained cells (grey columns) or Annexin V-binding assay (white columns). Results are the mean (±SD) of al least three independent experiments. (B) Annexin V-binding assay. Representative dot plots showing CPT83-treated (a) or untreated (b) GBM cells. Apoptotic cells are in the low right quadrant (Annexin V-positive, propidium iodide-negative), whereas necrotic cells are in the up-right quadrant (Annexin V- and propidium iodide-positive).

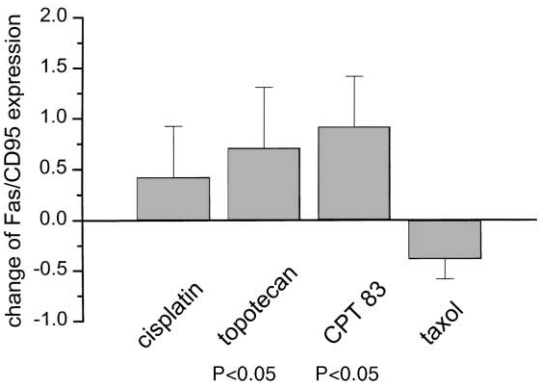

Fig. 2. Effect of 24 hr drug exposure on fluorescence intensity of anti-Fas/CD95-stained GBM cells. Cells were exposed to a drug concentration corresponding to the IC_{50}. Results are expressed as ratios of mean fluorescence intensity (MFI) obtained with specific antibody divided by fluorescence obtained with control antibody of treated and untreated cells. Results are the mean (±SD) of at least three independent experiments.

3.3. Fas/CD95 expression and drug-induced modulation

In our experimental conditions, GBM cells showed constitutive low expression of Fas/CD95. GBM cells were *in vitro* exposed to the various antitumour drugs and the results regarding Fas/CD95 expression are summarised in Fig. 2. A significant increase ($P < 0.05$) in Fas/CD95 expression was detected after 24 hr exposure to the topoisomerase I inhibitors (topotecan and CPT83) if compared to baseline levels in untreated cells. A trend to increase expression of Fas/CD95 was observed following cisplatin exposure. Treatment of GBM cells with taxol did not affect Fas/CD95 expression.

3.4. Susceptibility to Fas/CD95-mediated apoptosis

Untreated GBM cells did not exhibit a marked susceptibility to anti-Fas/CD95 antibody-mediated apoptosis as compared to Jurkat cells (Fig. 3). Indeed, the agonistic antibody slightly increased basal apoptosis only at late time points and reached 11% at 96 hr. This observation is consistent with low level of Fas/CD95 expression.

Since resistance to Fas/CD95-mediated apoptosis has been related to the expression of abnormal forms of Fas/CD95 receptor due to alternative splicing [14,15], an RT-PCR analysis of Fas/CD95 mRNA transcript was undertaken in GBM cells. The normal transcript from exon 8 was found in GBM cells (175 bp), whereas the truncated Fas/CD95 was not expressed. The soluble form of Fas/CD95 (Fas/CΔ95DTM) was slightly expressed. A quantitative analysis of its relative expression indicated that it represented about 10% of total transcript, similarly to what was observed in normal peripheral blood lymphocytes (12%) (Fig. 4).

To examine whether drug-induced up-regulation of Fas/CD95 resulted in increased susceptibility to Fas/CD95-dependent apoptosis, 24 hr after drug exposure, drug was

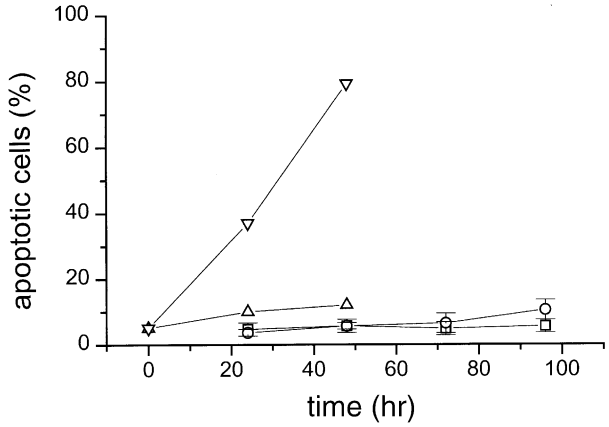

Fig. 3. Susceptibility of GBM cells to Fas/CD95-induced apoptosis. Apoptosis induction was evaluated after incubation of cells with an apoptosis inducing anti-Fas/CD95 antibody (1 μg/mL). At different times, apoptosis levels were measured through cytofluorimetric analysis of propidium iodide-stained cells. For GBM cells, the reported values are the mean (±SD) of five independent experiments. The Jurkat cell line was used as positive control (representative experiment). Untreated GBM cells (□); untreated Jurkat cells (△); antibody-treated GBM cells (○); antibody-treated Jurkat cells (▽).

removed and cells were incubated with an agonistic anti-Fas/CD95 antibody (1 μg/mL). At various time points, cells were analysed for monitoring apoptotic cell death. In the case of treatment with topotecan and CPT83, up-regulation of Fas/CD95 was associated with an increased susceptibility to Fas/CD95-mediated apoptosis (Fig. 5C and D, respectively), whereas this effect was not detected in cisplatin-treated cells (Fig. 5A). A marginal interaction was observed between anti-Fas/CD95 antibody and taxol (Fig. 5B). This finding is consistent with lack of Fas/CD95 up-regulation by taxol.

Potentiation of Fas/CD95-dependent apoptosis by topotecan has been related to inhibition of RNA synthesis [17]. Therefore, we measured RNA synthesis following 4 and

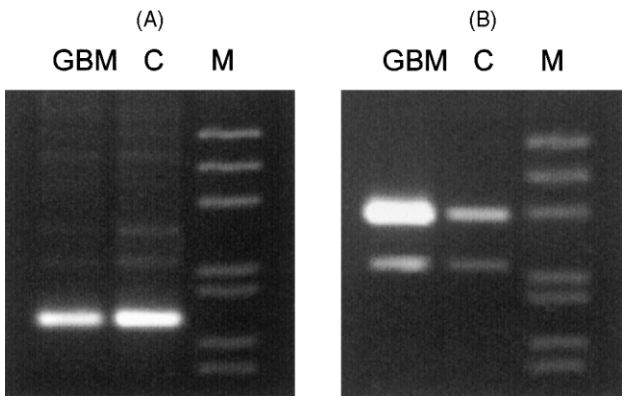

Fig. 4. Analysis of Fas/CD95 transcripts in GBM cells by RT-PCR. PCR products with primers for exon 8 deletion Fas/CD95 transcript are shown in A, whereas PCR products obtained with primers for Fas/CΔ95DTM transcripts are shown in B. Normal peripheral blood lymphocytes were used as control (C). M, marker, pBR322 *Hin*f-digested DNA.

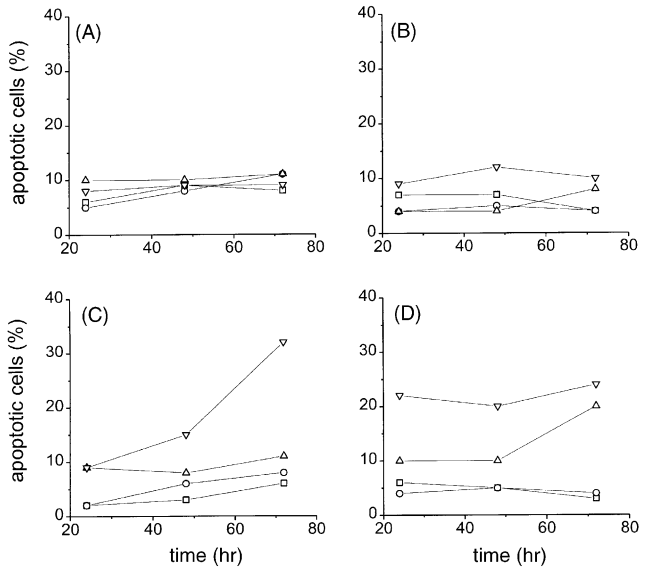

Fig. 5. Differential modulation of susceptibility to drug-induced apoptosis by anti-Fas/CD95 antibody. GBM cells were exposed to drug concentrations corresponding to the IC_{80} (0.3 μg/mL cisplatin; 0.1 μg/mL topotecan; 0.01 μg/mL CPT83; 0.1 μg/mL taxol) for 24 hr and then incubated in the absence or presence of the anti-Fas/CD95 antibody (1 μg/mL) for the indicated time. At different times, apoptosis levels were measured through cytofluorimetric analysis of propidium iodide-stained cells. Representative experiments with triplicate determinations are shown (SD < 10%). Untreated GBM cells (□); cells exposed to the antibody alone (○); drug-treated cells (△); cells exposed to both drug and antibody (▽). (A) Cisplatin, (B) taxol, (C) topotecan, (D) CPT83.

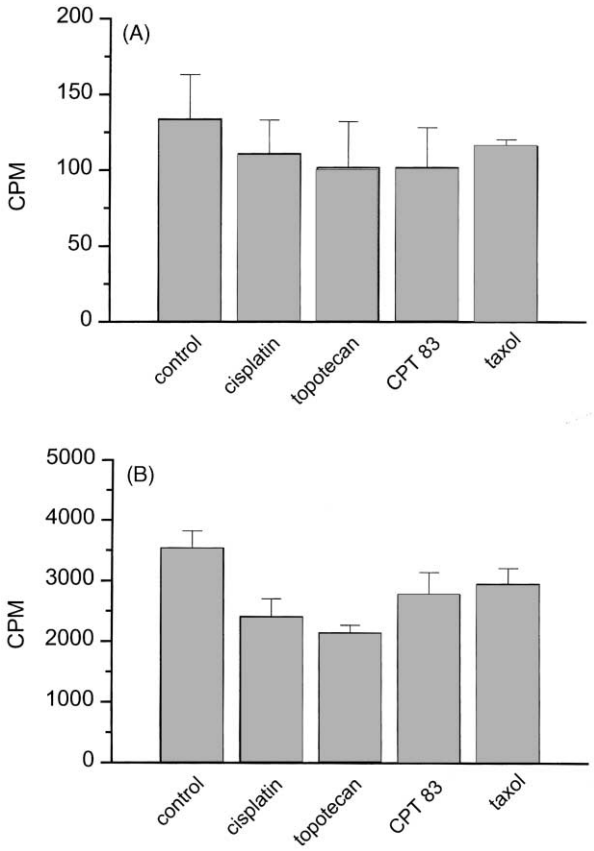

Fig. 6. Effects of cisplatin, topotecan, CPT83 and taxol on RNA synthesis. GBM cells were exposed to drug concentrations corresponding to the IC_{80} (0.3 μg/mL cisplatin; 0.1 μg/mL topotecan; 0.01 μg/mL CPT83; 0.1 μg/mL taxol) for 4 hr (A) or 24 hr (B) in the presence of ^{3}H-uridine. The reported values are the mean (±SD) of three independent experiments.

24 hr drug exposure (Fig. 6). Under these conditions, no statistically significant differences were observed between topoisomerase I inhibitors and other drugs. Therefore, no relationship between RNA synthesis inhibition and synergism was found.

4. Discussion

Apoptosis is regarded as the primary mode of cell death in antitumour response to effective antitumour drug [18,19]. An analysis of drug-induced apoptosis indicated that the GBM cell system was relatively resistant to activation of apoptotic response to conventional cytotoxic agents in spite of marked antiproliferative effects (Fig. 1). Indeed, the maximum level of drug-induced apoptotic cell death was around 15% and was achieved after exposure to CPT83. The relative resistance to apoptosis induction by DNA damaging agents (e.g. cisplatin) could be related, at least in part, to the loss of *p53* function following mutation. The loss of *p53* function was also documented by lack of Bax up-regulation following treatment (not shown). A number of alterations concerning the cell death pathways (including *p53* mutation and Bcl-2 overexpression) have been implicated in the resistance of malignant gliomas [20,21]. In spite of the apparently low susceptibility to drug-induced apoptosis, most of the high-grade gliomas are characterised by a significant expression of the death receptor Fas/CD95 [10,22]. Our study shows that the expression of Fas/CD95 in GBM glioma cells could be enhanced by specific DNA damaging agents, particularly by topoisomerase I inhibitors (Fig. 2). In the case of topotecan and CPT83, the drug-induced up-regulation of Fas/CD95 was concomitant to an increased susceptibility of GBM cells to Fas/CD95-dependent apoptosis (Fig. 5). A study of the pattern of cell sensitivity indicated that the GBM cell line was particularly sensitive to topoisomerase I inhibitors, suggesting the interest of these drugs in the treatment of gliomas (Table 1). The clinical interest of topotecan in malignant glioma remains to be documented [23]. The recent development of more lipophilic analogs is a promising approach in an attempt to improve the therapy of malignant gliomas with camptothecins [16,24]. A synergistic interaction of camptothecins with other DNA damaging agents (including cisplatin and ionising radiation) has been documented [25,26]. The present study further supports that modulation of Fas/CD95 by topoisomerase I inhibitors may be a valuable target for sensitisation of glioma cells. It has been reported that overexpression of Fas/CD95 is able to induce apoptosis in glioma cell lines

per se [27]. However, in our cell system drug-induced up-regulation of Fas/CD95 was not *per se* sufficient to induce apoptosis, thus, suggesting the co-operation among multiple pathways to induce an efficient apoptotic response.

In agreement with the results found in other glioma cell lines [17], cytotoxic concentrations (lower to or equal to IC_{80}) did not significantly affect RNA synthesis, therefore, inhibition of RNA synthesis could not explain the increased susceptibility of GBM cells to Fas/CD95-dependent apoptosis. In untreated GBM cells, susceptibility to Fas/CD95-mediated apoptosis was very low (Fig. 3) and apparently was not related to the expression of abnormal forms of Fas/CD95. Indeed, a molecular analysis of specific transcripts indicated that the truncated Fas/CD95 receptor which lacks the death signalling domain of the molecule was not expressed (Fig. 4). In addition, a low level of expression of soluble Fas/CD95, not containing the transmembrane domain was observed. Since the proportion of soluble Fas/CD95 found in the GBM cells was low (around 10% of total transcript), it is unlikely that competition of soluble Fas/CD95 with the normal protein completely account for the observed low sensitivity to Fas/CD95-mediated apoptosis.

The finding that Fas/CD95 was up-regulated in a cell line containing a mutant *p53* was not surprising considering the recent report showing that human Fas/CD95 responsive elements are activated by mutant forms of *p53* unable to induce apoptosis [28]. However, we did not detect up-regulation of *p53* levels following DNA damage (not shown), suggesting that the observed increase of Fas/CD95 is *p53*-independent. Although *p53* has been reported to up-regulate transcription of Fas/CD95 [29], the relation between drug-induced activation of *p53* and the CD95/CD95 ligand system may be dependent on the tumour cell type [30]. Again, it is likely that the relevance of *p53* status on the chemosensitivity is dependent on the type of cytotoxic injuries [31]. A plausible explanation for the remarkable sensitivity of *p53* mutant cells to DNA topoisomerase I inhibitors may be that the lack of G1 checkpoint following *p53* inactivation allows the formation of lethal double-strand DNA breaks during DNA replication [32]. Indeed, introduction of the mutant *p53* gene has been reported to enhance sensitivity of glioma cells carrying wild-type *p53* [17]. Taken together with the pattern of response of GBM cells to different DNA damaging agents, these observations suggest that, in contrast to platinum compounds, camptothecins are able to activate a *p53*-independent apoptosis pathway. A tentative explanation for the potentiation of apoptotic response by anti-Fas antibody only with camptothecins (but not cisplatin or taxol) may be a synergistic interaction between multiple pathways resulting in cell death. In the case of cisplatin, the loss of functional *p53* might prevent an efficient cell death pathway in spite of a trend towards Fas/CD95 induction by the drug. Conversely, the lack of induction of Fas/CD95 by taxol might be a limiting factor for an efficient apoptotic response, in spite of ability of the taxane to activate a *p53*-independent apoptosis.

In conclusion, the most relevant finding of this study is the observation that some DNA damaging agents are capable of inducing up-regulation of Fas/CD95 in a *p53* mutant cell system. A synergistic interaction was observed between camptothecins and anti-Fas antibody. No sensitisation to apoptosis was obtained with taxol which did not up-regulate Fas/CD95 receptor. Cisplatin was capable of up-regulating Fas/CD95 receptor expression although at a non-significant level, but failed to make the cells more prone to Fas/CD95-mediated apoptosis. Thus, whereas Fas/CD95-up-regulation appeared to be a common response to DNA damage, it is likely that the specific *p53*-independent pathway activated by topoisomerase I inhibitors converges on Fas-dependent pathways resulting in an enhanced cell death. The activation of Fas/CD95-dependent pathway was not *per se* sufficient to induce an efficient apoptotic response as indicated by a lack of modulation of caspase 8 following camptothecin treatment in absence of anti-Fas antibody exposure (not shown).

The evidence that glioma cells express Fas/CD95-dependent cell death pathway [21] and the Fas/CD95 receptor may be modulated by topoisomerase I inhibitors may have pharmacological implications for the design of novel therapeutic strategies in the treatment of malignant gliomas. The pharmacological interest of modulation of cell death receptors is also supported by a recent preclinical study on *in vivo* combination of irinotecan and TRAIL [33]. The availability of lipophilic camptothecins like CPT83 and analogs of this series able to penetrate the blood-brain barrier could allow evaluation of efficacy of such combinations in intracranial tumours.

Acknowledgments

This work was partially supported by the Associazione Italiana per la Ricerca sul Cancro, Milan, by the Ministero della Sanita', Rome, Italy, and by the Consiglio Nazionale delle Ricerche, Rome, Italy. The authors wish to thank Maurizio Gelati for technical assistance.

References

[1] Shapiro WR, Shapiro JR. Biology and treatment of malignant glioma. Oncology 1998;12:233–40.
[2] Weller M, Rieger J, Grimmel C, Van Meir EG, De Tribolet N, Krajewski S, Reed JC, Von Deimling A, Dichgans J. Predicting chemoresistance in human malignant glioma cells: the role of molecular genetic analyses. Int J Cancer 1998;79:640–4.
[3] Naumann U, Weller M. Retroviral *BAX* gene transfer fails to sensitize malignant glioma cells to CD95L-induced apoptosis and cancer chemotherapy. Int J Cancer 1998;77:645–8.
[4] Orlinick JR, Vaishnaw AK, Elkon KB. Structure and function of Fas/Fas ligand. Int Rev Immunol 1999;18:293–308.

[5] Watanabe-Fukunaga R, Brannan CI, Copeland NG, Jenkins NA, Nagata S. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. Nature 1992;356:314–7.
[6] Wang D, Freeman GJ, Levine H, Ritz J, Robertson MJ. Role of the CD40 and CD95 (APO1/Fas) antigens in the apoptosis of human B-cell malignancies. Br J Haematol 1997;97:409–17.
[7] Kalechman Y, Strassmann G, Albeck M, Sredni B. Up-regulation by ammonium trichloro(dioxoethylene-0,0′) tellurate (AS101) of Fas/Apo1 expression on B16 melanoma cells: implications for the antitumor effects of AS101. J Immunol 1998;161:3536–42.
[8] Xu X, Fu XY, Plate J, Chong AS. IFN-gamma induces cell growth inhibition by Fas-mediated apoptosis: requirement of STAT1 protein for up-regulation of Fas and FasL expression. Cancer Res 1998;58:2832–7.
[9] Frigerio S, Silei V, Ciusani E, Massa G, Lauro GM, Salmaggi A. Modulation of Fas-ligand (Fas-L) on human microglial cells: an *in vitro* study. J Neuroimmunol 2000;105:109–14.
[10] Tachibana O, Nakazawa H, Lampe J, Watanabe K, Kleihues P, Ohgaki H. Expression of Fas/APO1 during the progression of astrocytomas. Cancer Res 1995;55:5528–30.
[11] Perego P, Boiardi A, Carenini N, De Cesare M, Dolfini E, Giardini R, Magnani I, Martignone S, Silvani A, Soranzo C, Zunino F. Characterization of an established human, malignant, glioblastoma cell line (GBM) and its response to conventional drugs. J Cancer Res Clin Oncol 1994;120:585–92.
[12] Spinozzi F, Agea E, Bistoni O, Travetti A, Migliorati G, Moraca R, Nicoletti I, Riccardi C, Paoletti FP, Vaccaro R, Bertotto A. T lymphocytes bearing the gamma delta T cell receptor are susceptible to steroid-induced programmed cell death. Scand J Immunol 1995;41:504–8.
[13] Perego P, Giarola M, Righetti SC, Supino R, Caserini C, Delia D, Pierotti MA, Miyashita T, Reed JC, Zunino F. Association between cisplatin resistance and mutation of *p53* gene and reduced *Bax* expression in ovarian carcinoma cell systems. Cancer Res 1996;56:556–62.
[14] Rokhlin OW, Bishop GA, Hostager BS, Waldschmidt TJ, Sidorenko SP, Pavloff N, Kiefer MC, Umansky SR, Glover RA, Cohen MB. Fas-mediated apoptosis in human prostatic carcinoma cell lines. Cancer Res 1997;57:1758–68.
[15] Cascino I, Papoff G, De Maria R, Testi R, Ruberti G. Fas/Apo1 (CD95) receptor lacking the intracytoplasmic signaling domain protects tumor cells from Fas-mediated apoptosis. J Immunol 1996;156:13–7.
[16] Dallavalla S, Delsoldato T, Ferrari A, Merlini L, Penco S, Carenini N, Perego P, De Cesare M, Pratesi G, Zunino F. Novel 7-substituted camptothecins with potent antitumor activity. J Med Chem 2000;43:3963–9.
[17] Winter S, Weller M. Potentiation of CD95L-induced apoptosis of human malignant glioma cells by topotecan involves inhibition of RNA synthesis but not changes in CD95 or CD95L protein expression. J Pharmacol Exp Ther 1998;286:1364–82.
[18] Zunino F, Perego P, Pilotti S, Pratesi G, Supino R, Arcamone F. Role of apoptotic response in cellular resistance to cytotoxic agents. Pharmacol Ther 1997;76:177–85.
[19] Hickman JA. Apoptosis and chemotherapy resistance. Eur J Cancer 1996;32:921–6.
[20] Rempel SA. Molecular biology of central nervous system tumors. Curr Opin Oncol 1998;10:179–85.
[21] Nieder C, Petersen S, Petersen C, Thames HD. The challenge of *p53* as prognostic and predictive factor in gliomas. Cancer Treat Rev 2000;26:67–73.
[22] Weller M, Frei K, Groscurth P, Krammer PH, Yonekawa Y, Fontana A. Anti-Fas/APO1 antibody-mediated apoptosis of cultures human glioma cells: induction and modulation of sensitivity by cytokines. J Clin Invest 1994;94:954–65.
[23] Schmidt F, Schuster M, Streffer J, Schabet M, Weller M. Topotecan-based combination chemotherapy for human malignant glioma. Anticancer Res 1999;19:1217–22.
[24] Pollack IF, Erff M, Bom D, Burke TG, Strode JT, Curran DP. Potent topoisomerase I inhibition by novel silatecans eliminates glioma proliferation *in vitro* and *in vivo*. Cancer Res 1999;59:4898–905.
[25] Romanelli S, Perego P, Pratesi G, Carenini N, Tortoreto M, Zunino F. *In vitro* and *in vivo* interaction between cisplatin and topotecan in ovarian carcinoma systems. Cancer Chemother Pharmacol 1998;41:385–90.
[26] Marchesini R, Colombo A, Caserini C, Perego P, Supino R, Capranico G, Tronconi M, Zunino F. Interaction of ionizing radiation with topotecan in two human tumor cell lines. Int J Cancer 1996;66:342–6.
[27] Shinoura N, Ohashi M, Yoshida Y, Kirino T, Asai A, Hashimoto M, Hamada H. Adenovirus-mediated overexpression of Fas induces apoptosis of gliomas. Cancer Gene Ther 2000;7:224–32.
[28] Munsch D, Watanabe-Fukunaga R, Bourdon J-C, Nagata S, May E, Yonish-Rouach E, Reisdorf P. Human and mouse *Fas* (*APO1/CD95*) death receptor genes each contain a *p53*-responsive element that is activated by *p53* mutants unable to induce apoptosis. J Biol Chem 2000;275:3867–72.
[29] Kastan M. On the trail from *p53* to apoptosis? Nat Genet 1997;17:130–1.
[30] Weller M. Predicting response to cancer chemotherapy: the role of *p53*. Cell Tissue Res 1998;292:435–45.
[31] Roth W, Wagenknecht B, Grimmel C, Dichgans J, Weller M. Taxol-mediated augmentation of CD95 ligand-induced apoptosis of human malignant glioma cells: association with *bcl-2* phosphorylation but neither activation of *p53* nor G2/M cell cycle arrest. Br J Cancer 1998;77:404–11.
[32] Pommier Y, Pourquier P, Fan Y, Strumberg D. Mechanism of action of eukaryotic DNA topoisomerase I and drugs targeted to the enzyme. Biochim Biophys Acta 1998;1400:83–105.
[33] Gliniak B, Le T. Tumor necrosis factor-related apoptosis-inducing ligand's antitumor activity *in vivo* is enhanced by the chemotherapeutic agent CPT-11. Cancer Res 1999;59:6153–8.