Acta Neurochir (Wien) (2004) 146: 721–729
DOI 10.1007/s00701-004-0286-4

Acta Neurochirurgica
Printed in Austria

Experimental Research

Cell death induction by betulinic acid, ceramide and TRAIL in primary glioblastoma multiforme cells

I. Jeremias[1], **H. H. Steiner**[2], **A. Benner**[3], **K.-M. Debatin**[4], and **C. Herold-Mende**[2,5]

[1] Dr. von Haunersches Kinderspital, Munich, Germany
[2] Department of Neurosurgery, Section Molecular Biology, University Heidelberg, Heidelberg, Germany
[3] Central Unit Biostatistics, German Cancer Research Center, Heidelberg, Germany
[4] University Clinic and Policlinic for Children and Adolescents, Ulm, Germany
[5] Department of Head and Neck Surgery, University Heidelberg, Heidelberg, Germany

Published online May 21, 2004

Summary

Background. Glioblastoma multiforme (WHO Grade IV, GBM) is the most malignant brain tumour with a mean survival time of less than one year. Betulinic acid, ceramide and TRAIL (TNF-related apoptosis inducing ligand) represent novel therapeutic agents for potential use in GBM.

Method. Primary GBM cells of 21 patients with macroscopically complete tumour resection were tested in vitro for cell death induction by betulinic acid, ceramide, TRAIL and established therapeutics (BCNU, cisplatin, doxorubicin, vincristin and γ-irradiation).

Findings. At peak plasma concentrations (PPC), Betulinic acid, ceramide and TRAIL induced cell death in primary GBM cells at higher rates than established cytotoxic drugs. Specific cell death $\geq$75% was observed in 43% (9/21), 38% (8/21), and 19% (4/21) for betulinic acid, ceramide, and TRAIL respectively, while this was only found in 5% (1/21) of γ-irradiated and cisplatin-treated cells, and in none of the GBM cultures, where BCNU or vincristin were applied in PPC.

Conclusion. Due to a markedly improved cell death of GBM cells as compared with established therapeutics, Betulinic acid, ceramide and TRAIL might represent potent substances for future treatment of GBM.

Keywords: Glioblastoma multiforme WHO IV; betulinic acid; ceramide; TRAIL.

Introduction

Malignant neoplasm of the brain represents the second leading cause of cancer-related mortality in children under the age of 15 and the fourth leading cause in adults between the age of 15 and 34. The most malignant form, glioblastoma multiforme (WHO Grade IV, GBM), occurs in 2–3 new cases per 100 000 population per year. Even after careful surgical excision and despite multimodal treatment including radio- and chemotherapy, prognosis of patients with GBM is poor, with a median survival time of only 1 year and survival rates of 15% and 2% after 2 and 3 years, respectively [19]. Prognostic factors include age, physical condition (''Karnofsky Index'') and macroscopically complete resectability of the tumour [17, 21]. The diffuse infiltrative growth pattern of single tumour cells leads to postoperative local regrowth in all patients. Until today, GBM remains a rapidly progressive, lethal tumour and improved treatment is desirable.

Potential new therapeutic substances for future GBM treatment include betulinic acid (BA), ceramide and TNF-related apoptosis inducing ligand (TRAIL, also called Apo-2L). BA is prepared from birch trees and was shown to induce cell death independently from ligand-receptor interaction by directly activating mitochondria [4] thus directly activating the intrinsic apoptosis signalling pathway. Ceramide is part of the sphingolipid metabolism and found as second messenger in various apoptosis pathways [10]. TRAIL is a member of the TNF-family together with, TNFα (Tumor necrosis factor α) and CD95-L (CD95-Ligand, also called Apo-1 Ligand or Fas-Ligand). TRAIL mediates cell death via its specific receptors TRAIL-R1 (TRAIL-receptor 1) and TRAIL-R2 [1, 15, 16], e.g. in GBM [5]. Intracellularly upon activation, FADD (Fas-associated polypeptide with

death domain) is recruited to the TRAIL-receptors and activates caspases. Activation of caspases is either direct or mediated by mitochondria and the apoptosome. All three substances show little toxicity when tested in animal models [20, 23] and are considered as potential future cytotoxic drugs.

In the present study, we were interested in investigating new compounds with promising characteristics and potential anti-tumour activity for GBM. We choose BA, ceramide and TRAIL as these substances are untoxic in animal trials and a systematic comparison to established therapeutics is still lacking. We tested BA, ceramide and TRAIL on primary GBM tumour cultures and compared cell death efficiency with those of conventional cytotoxic drugs and γ-irradiation.

Methods

Study design

In a retrospective study, patient data were obtained from clinical data sheets. Primary cells were cultured from fresh tumour material, frozen, thawed, and treated with various stimuli in vitro. Cell death was measured in a blinded fashion and its correlation with clinical data was analysed.

Patients

Patients were operated on in the Neurosurgery Department of the University Hospital in Heidelberg during a 2.5 year period and informed consent was obtained for this study. Diagnosis of GBM was based on the histochemical evaluation by the neuropathologist.

Inclusion criteria for this study were:

- glioblastoma multiforme following WHO-criteria diagnosed by the neuropathologist
- single tumour site, no metastasis
- tumour resection directly after diagnosis without prior adjuvant therapy
- macroscopically complete tumour resection estimated by the surgeon
- macroscopically complete tumour resection estimated by MRI on day 1 after surgery
- routine MRI scan every 3 month after surgery
- fractionated radiotherapy of 60 Gy (3 Gy 3 times weekly)
- complete clinical follow up

Materials

Doxorubicin, BCNU, BA and cisplatin were purchased by Sigma (Deisenhofen, FRG), vincristin from USPC Inc (Rockville, MD), TNFα from Calbiochem (San Diego, CA), Ceramide from Biochrom (Berlin, FRG). The TRAIL protein was produced in a recombinant expression system using the yeast Pichia Pastoris [8]. In brief, Pichia Pastoris was transformed by cloned His-tagged TRAIL-DNA under the AOX-1 promoter. Induction of protein expression was achieved by addition of methanol. Thereafter, cells were lysed and TRAIL protein was purified using Nickel-Histidine interaction [8]. All further agents were obtained from Sigma (Deisenhofen, FRG). Apart from TNFα, APO-1 and TRAIL, all agents were dissolved directly prior to each experiment.

Culture of primary GBM cells

Primary GBM cells were cultured as described elsewhere [6] by dissecting tissue in small pieces of about 1 mm^3 and transferring in 75 cm^2 plastic culture flasks (Falcon, Becton Dickinson, Heidelberg, Germany). Cells were cultured routinely in RPMI 1640 (Life Technologies, Eggenstein, Germany) supplemented by 10% heat-inactivated fetal calf serum (Conco, Wiesbaden, Germany), 2 mM L-glutamine (Biochrom, Berlin, Germany) and antibiotics at 37 °C, 5% CO_2 and 95% air in a humidified incubator with medium changes twice a week. After reaching confluency, cells were harvested by a brief incubation with trypsin/EDTA solution (Viralex, PAA, Linz, Austria) and seeded into a fresh 75 cm^2 plastic tissue culture flask. Doubling time varied between 2 to 5 days. Tumour cells were characterised for their astrocytic origin by immunohistochemical detection of the tissue specific marker GFAP. Only cell cultures showing a homogenous staining for GFAP marker were used in this study. Tumour cell cultures were free of endothelial cell contamination as determined by staining with endothelial cell specific antibodies against both factor VIII and PECAM-1. Cells were frozen after a mean of 5 passages and were kept at −190 °C. For this study, cells were thawed and stimulation was performed after 3 passages of culture as described above.

In vitro stimulation

10^5 cells/well were seeded on 24 well-plates in 500 µl medium for 24 hours. Stimulants were added for the complete incubation time in the following concentrations: BCNU 0.1, 1, 10, 100 µg/ml, doxorubicin 0.1, 0.3, 1 µg/ml, cisplatin 1, 10, 100 µg/ml, vincristin 0.1, 1, 10 µg/ml, APO-1 1 µg/ml in the presence of Protein A 5 ng/ml, TRAIL 0.06, 0.2, 0.6 µg/ml, TNF-α 0.1 µg/ml, BA 3, 10, 30, 100 µg/ml and ceramide 20, 60, 200, 600 nM in medium. Apo-1 and TNFα were used with and without the presence of CHX (cycloheximide) 0.5 µg/ml. γ-irradiation (60 Gy) was performed by exposure of cells for different time periods to caesium 137 using the Gamma-cell-count by Atomic Energy of Canada Limited. The substances tested activate the cell death pathway at different steps, namely membrane bound receptors (DILs) or directly (BA) or indirectly (rest) at the mitochondria. Unstimulated control cells were treated in exactly the same way as stimulated cells, except that PBS was added instead of the various stimuli. Incubation period was 24 hours for Apo-1, TRAIL, TNFα, BA and ceramide. For BCNU and doxorubicin incubation time was prolonged to 48 hours, for γ-irradiation to 72 hours given the kinetic of cell death induction by these stimuli. Cells were removed at the end of the experiment using Trypsin digestion and stained with Trypan blue directly before cell counting. At least 250 cells were counted for each test and evaluated for Trypan blue exclusion. Percentage of viable cells was calculated as [Trypan blue excluding cells divided by all cells times 100], cell death was calculated as [100 minus percentage of viable cells]. Propidium iodide staining was performed as described [11]. Data are the mean of at least duplicates of at least two independent experiments. Specific cell death was calculated as [(percentage of cell death of treated cells minus percentage of cell death of untreated cells) divided by (100 minus percentage of cell death of untreated cells) times 100]. Positive values show specific cell death, and if observed cell death is less than or equal to cell death of untreated cells then this number is less than or equal 0. The maximum value is 100, the minimum value depends on the cell death of untreated cells.

Statistical analysis

As a summary measure of specific cell death measured for different concentrations of in vitro stimulants, we used the area under the dose-response curve (AUC). We used the trapezoidal rule to compute the AUC. Spearman's rank correlation coefficient was computed to estimate pairwise correlations of in vitro and in vivo variables. All correlations

Table 1. *Clinical data of patients and cell death induction in primary GBM cells in vitro*

Number of patient	Clinical data				In vitro data							
	Age (years)	Gender	Time until tumour regrowth (months)	Time of survival (months)	Betulinic acid	Ceramide	TRAIL	BCNU	Cisplatin	Doxorubicin	Vincristin	γ-irradiation
NCH42	25	f	>72	>72	YES	no	no	no	no	YES	YES	YES
NCH47	47	f	15	40	YES	YES	no	YES	YES	YES	YES	YES
NCH52	22	m	12	30	YES	YES	no	no	YES	YES	YES	no
NCH59	45	f	6	18	YES	YES	no	YES	YES	YES	YES	YES
NCH60	57	m	9	19	no	YES	no	no	YES	YES	YES	no
NCH90	39	m	12	22	YES	no	YES	no	no	no	no	no
NCH92	72	f	12	18	no	YES	no	no	no	YES	no	no
NCH94	62	m	9	16	YES	no	YES	no	no	no	no	YES
NCH98	55	m	6	20	YES	no	YES	no	no	YES	no	YES
NCH105	27	m	18	35	YES	no	YES	YES	no	no	no	YES
NCH111	75	m	9	15	YES	no	no	no	no	YES	YES	no
NCH123	45	m	3	21	YES	YES	YES	YES	YES	YES	YES	no
NCH132	41	m	21	30	no	YES	no	no	no	YES	YES	YES
NCH139	45	m	12	29	no	no	no	no	no	no	no	YES
NCH141	67	f	9	13	no	YES	no	no	no	no	no	no
NCH145	37	f	3	5	no	YES	no	no	no	YES	no	no
NCH146	60	m	3	10	YES	YES	no	no	YES	YES	no	no
NCH149	64	m	12	16	no	YES	no	no	no	no	no	YES
NCH160	49	f	12	12	no	YES	no	no	no	no	no	no
NCH162	57	f	15	18	YES	YES	no	no	no	YES	YES	YES
NCH164	20	f	24	35	YES	YES	no	no	YES	YES	YES	no

Age age at 1. operation, *time until tumour regrowth* time from 1. operation until tumour regrowth was diagnosed by 3-monthly MRI, *time of survival* time from 1. operation until death, *f* female, *m* male. 21 primary GBM cells were stimulated in vitro as described in Methods. YES indicates ≥25%, no <25% specific cell death induced by betulinic acid 10 μg/ml, ceramide 60 nM, TRAIL 60 ng/ml, peak plasma concentration for cytotoxic drugs (BCNU 2 μg/ml, cisplatin 2 μg/ml, doxorubicin 0.3 μg/ml, vincristin 0.1 μg/ml) and γ-irradiation 60 Gy.

were calculated for patient data only. To identify possible prognostic in vitro variables regression analysis of survival data was performed by Cox's proportional hazards model [2] including the patients age as the only additional risk factor. Due to the small sample size of the study this was done for each in vitro variable individually.

Results

Patients

A total of 59 patients with GBM were operated on between March 1995 and October 1997 in the Neurosurgical department of the University of Heidelberg and were considered for this study. No anti-tumour therapy was applied before surgery. In 26 patients, tumour resection was macroscopically complete as confirmed both by the surgeon and by postoperative MRI scan. In 2 patients, clinical follow up data were incomplete. Cell culture of primary GBM cells was successful in 86.3% of samples during the study period so that complete in vivo and in vitro data were obtained from 21 patients who were included in the study. Postoperatively, patients received fractionated radiotherapy of 60 Gy and were monitored by MRI scan every 3 months. In the case of tumour regrowth, surgical resection was performed followed by chemotherapy using BCNU.

Patients had a median age of 48 years and gender distribution was female:male = 9:12 (Table 1). After 12, 24 and 36 months, 18, 7 and 2 out of 21 patients were alive, respectively. Corresponding survival estimates at 12, 24, and 36 months are 85.7%, 33.3%, and 9.5%, respectively. In line with published results, time of tumour regrowth was positively correlated with survival time (Spearman's $\rho = 0.69$; $p < 0.001$). Age was a statistically significant prognostic factor for survival ($p = 0.001$) and time of tumour regrowth ($p = 0.01$). Age was further negatively correlated with the Karnofsky index (Spearman's $\rho = -0.50$; $p = 0.02$).

Cell death induction in vitro

Cell viability was measured using Trypan blue staining throughout the study and cell death was calculated thereof. Propidium Iodide staining of DNA length [11]

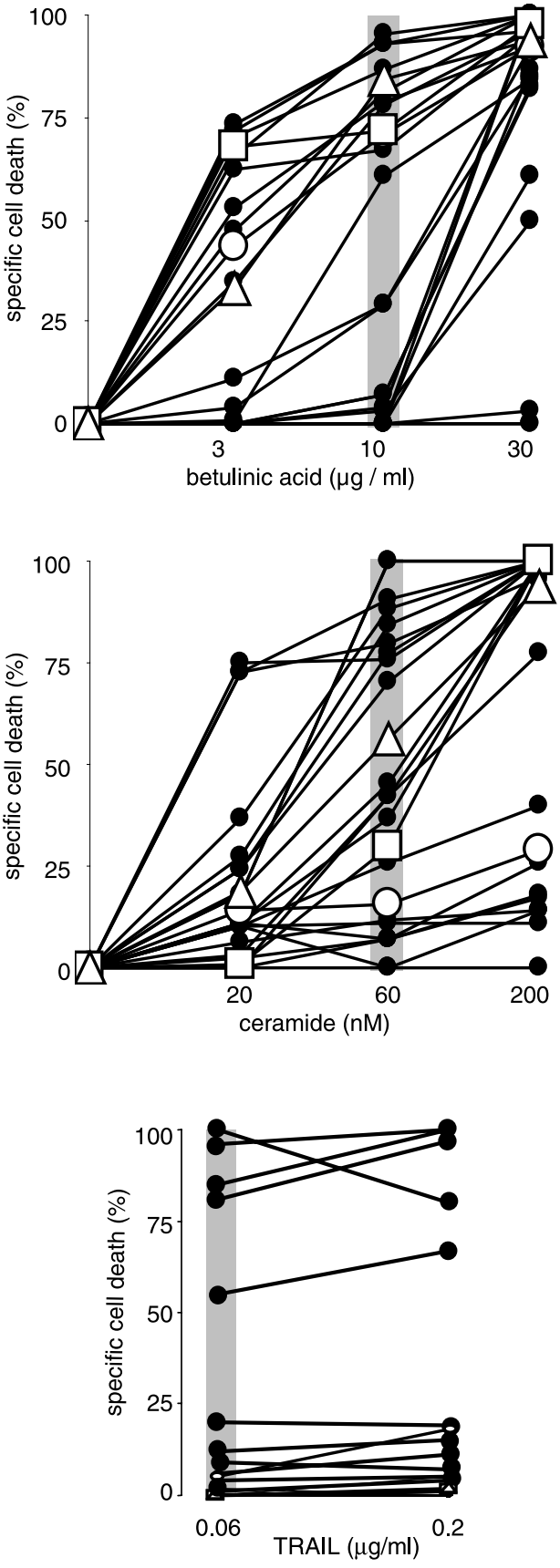

Fig. 1. Cell death induction in vitro. 21 primary GBM cell cultures and three established cell lines (U87MG open circle, A172 open square, U373MG open triangle) were stimulated as indicated for 24 hrs (betulinic acid, ceramide and TRAIL), 48 hrs (BCNU, cisplatin, doxorubicin and vincristin) or 72 hrs (γ-irradiation) hours, cell death was measured using Trypan blue exclusion and specific cell death was calculated as described in Methods. Grey bar = concentration used for comparison (plasma peak concentration for cytotoxic drugs)

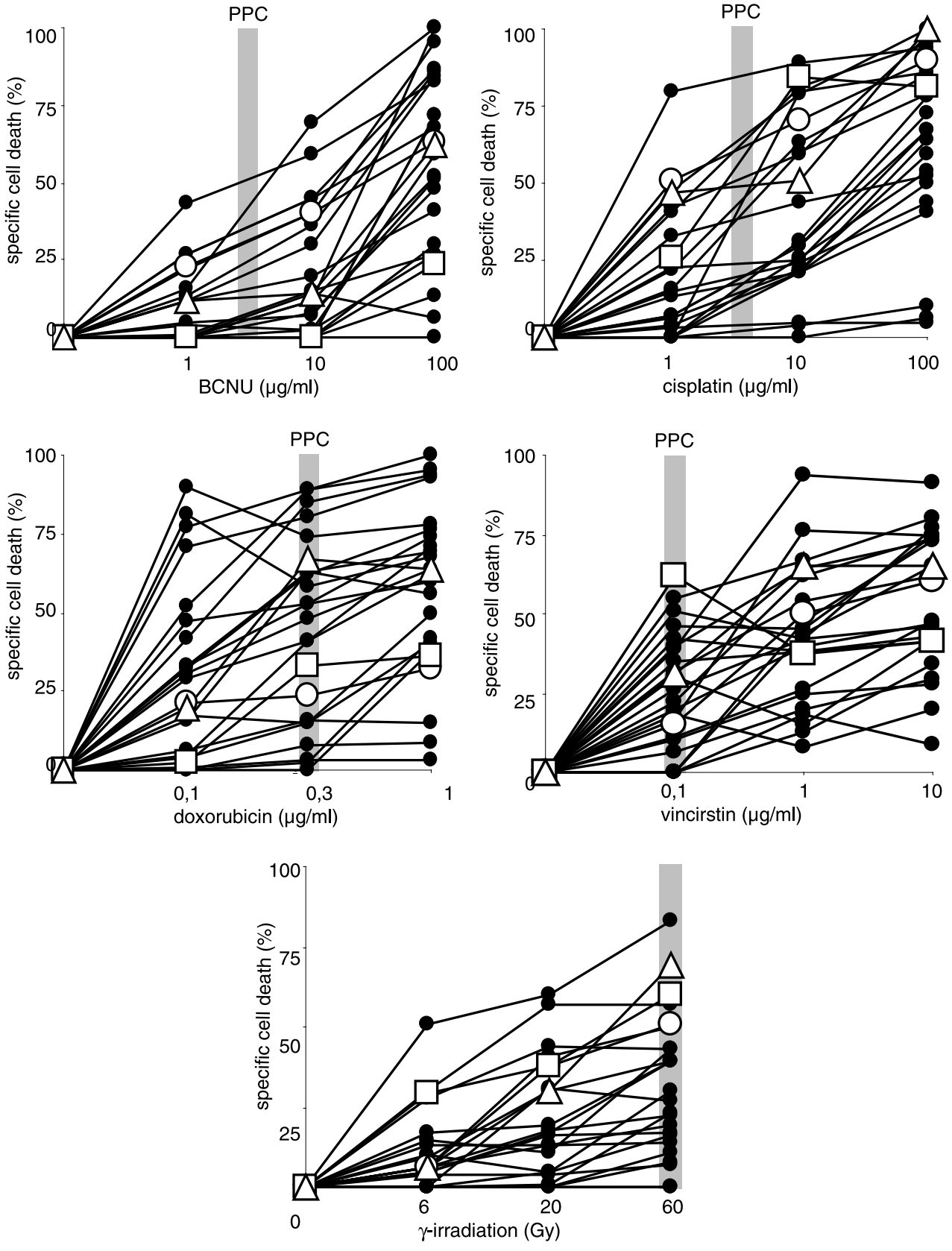

Fig. 1 (*continued*)

was performed in parallel for 5 primary GBM cultures and 2 established cell lines. For all stimuli and all concentrations used in the study, results obtained by both techniques were similar (data not shown). The frequently used MTT assay was not reliable in our hands due to high intra- and inter-assay-variances (data not shown).

For all substances tested, primary GBM cells showed a wide range of sensitivity and resistance towards cell death induction in vitro (Fig. 1). The three established cell lines used performed within the average of the primary GBM cells. For TRAIL, a dichotomy was found between primary samples highly sensitive (>50% specific cell death) and poorly sensitive (<25% specific cell death) for cell death induction. BA was found both a highly and widely active cell death inducing substance with more than 50% specific cell death in 90% (19/21) of samples. Sensitivity of primary tumour cells towards cell death induction by all agents tested differed broadly among patients (Table 1) according to the known heterogeneity of GBM [19]. Some primary GBM cells showed sensitivity (>25% specific cell death) towards most substances tested, e.g. NCH47, while in other samples, BA, ceramide and/or TRAIL were able to induce cell death in primary GBM cells completely resistant towards cytotoxic drugs and γ-irradiation (e.g. NCH90, NCH 141, NCH 160).

Correlation of cell death induction in vitro

The area under the ''dose-response'' curve (AUC) was calculated to represent cell death induction depending

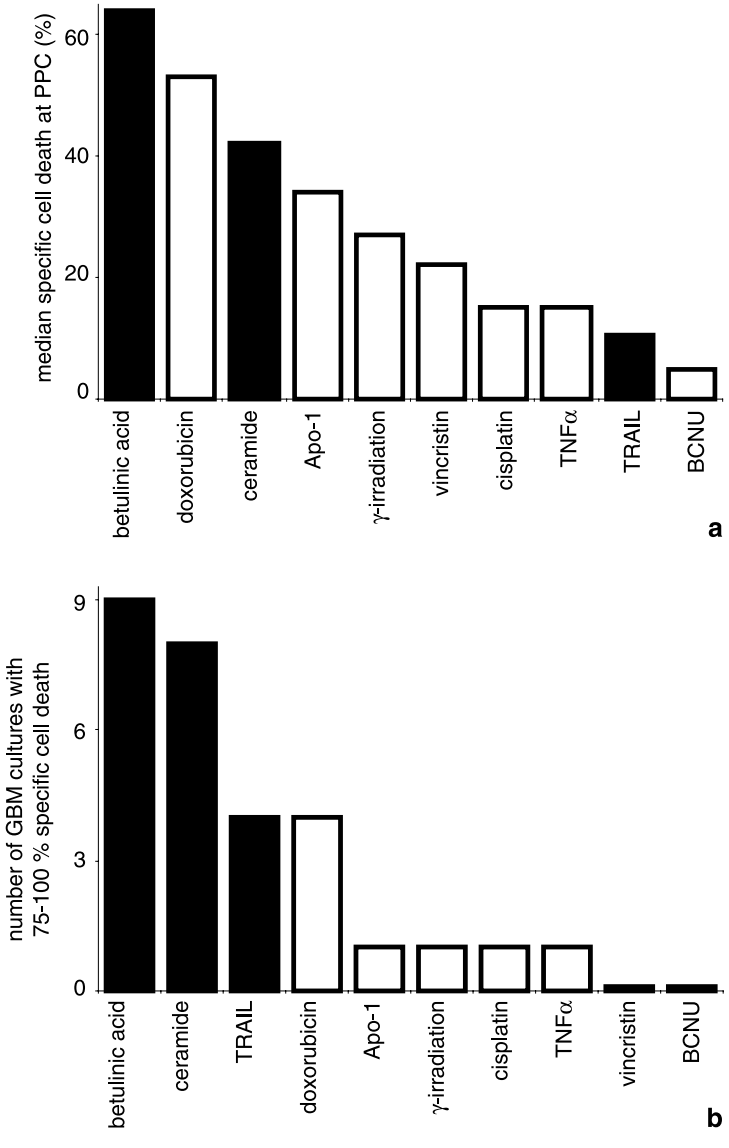

Fig. 2. Betulinic acid and ceramide induce cell death more effectively than cytotoxic drugs and γ-irradiation. Primary GBM cell cultures were stimulated as described in Methods. Shown is the median specific cell death (**a**) and number of primary GBM cultures undergoing $\geq$75% specific cell death (**b**) after stimulation with cytotoxic drugs at plasma peak concentration (2 µg/ml for BCNU and cisplatin, 0.3 µg/ml for doxorubicin and 0.1 µg/ml for vincristin), γ-irradiation 60 Gy, betulinic acid 10 µg/ml, ceramide 60 nM, TRAIL 60 ng/ml, Apo-1 1 µg/ml in the presence of Protein A 5 ng/ml and TNFα 0.1 µg/ml

on the various concentrations tested. TNFα in the presence of CHX correlated to cell death induction by Apo-1 ($\rho = 0.71$, $p < 0.001$) and TRAIL ($\rho = 0.47$, $p = 0.03$). Cell death induction by Apo-1 correlated to cell death induction by doxorubicin ($\rho = 0.50$, $p = 0.02$). When AUCs of different drugs were compared, a correlation was found between cell death induction by BCNU and vincristin ($\rho = 0.68$, $p < 0.001$), cisplatin and doxorubicin ($\rho = 0.74$, $p < 0.001$) and cisplatin and vincristin ($\rho = 0.55$, $p = 0.01$) suggesting common intracellular apoptosis signalling molecules. In vitro sensitivity of primary GBM cells to BA or ceramide did not show correlation with sensitivity for any further substance tested.

Betulinic acid and ceramide induce cell death more effectively than cytotoxic drugs and γ-irradiation

For comparison of cell death efficiency between the different substances tested, γ-irradiation was used at 60 Gy which resembles the therapeutic dose given to all patients. Cytotoxic drugs were evaluated at plasma peak concentration (PPC) to approximate the clinical situation (2 µg/ml for BCNU and cisplatin, 0.3 µg/ml for doxorubicin and 0.1 µg/ml for vincristin) [12–14, 18]. BA was nontoxic in animal studies in concentrations up to 100 mg/kg body weight [23] equal to at least 100 µg/ml ml PPC so that 10 µg/ml were chosen for comparison. For TRAIL, peak plasma concentrations directly after intravenous injection was 10 µg/ml in mouse, rat and chimpanzee and 100 µg/ml in Cynomolgus monkey, decreasing to 60 ng/ml within 4 hours [9] so that 60 ng/ml were chosen for comparison, while ceramide was evaluated at 60 nM. At PPC, all clinically established therapeutics including BCNU and γ-irradiation were found poor inducers of cell death in vitro except doxorubicin, which significantly induced cell death. In contrast, both betulinic and ceramide were found more potent for inducing cell death in primary GBM cells in vitro than the cytotoxic drugs tested in parallel (Fig. 2A). On average, BA induced more than 60% specific cell death, while most drugs did so only in about 20%. Including TRAIL, all three new agents were able to induce high cell death rates of 75–100% primary tumour cell cultures, e.g. betulinic acid in 9 (43%) samples, while drugs did so only in 0 to 4 (19%) samples (Fig. 2B).

Discussion

Death inducing ligands (TRAIL, TNFα and APO-1/CD95/Fas) and death receptor-independent substances (BA, ceramide) have stimulated interest as new antineoplastic agents. BA is nontoxic in animal trials in high concentrations and especially active against neuroectodermal tumours [23]. Ceramide can induce cell death in otherwise apoptosis-resistant tumours as it acts as second messenger in various apoptosis pathways [10]. TRAIL represents a promising future anti-cancer drug as it effectively induces cell death in tumour cells, but not in normal cells such as neurons [20] without toxicity in animal trials including monkeys [1]. TRAIL is active against GBM in vivo especially in the context of polychemotherapy [5]. BA, ceramide and TRAIL induced cell death in GBM cell lines in vitro and phase I clinical trails are under way or in preparation. As all three substances represent putative future cytotoxic drugs to treat GBM, we tested the in vitro efficacy of these substances on primary GBM tumour cultures in comparison with established therapeutics such as BCNU, vincristin and γ-irradiation.

We studied the tumour cells of 21 patients suffering GBM who received macroscopically complete tumour resection followed by radiotherapy and 3-monthly MRI-diagnostics. Although representing a small collection, the clinical data of our patients reflect the well known dependence on age, time until tumour regrowth and time of survival. The analysis of this representative panel of GBM cultures revealed BA and ceramide as most effective substances in vitro compared with all conventional treatment strategies analysed. This is supported by the observation that the application of frequently used therapeutics in GBM therapy such as BCNU, vincristin, and γ-irradiation leads only in at most 5% of the cases analysed a specific cell death $\geq$75%, while this was seen in 43%, 38%, and 19% for BA, ceramide, and TRAIL respectively. This is particularly interesting since we have chosen concentrations well below the plasma peak concentration found in animal trials, e.g. factor 1000 for TRAIL [9] as no plasma peak concentration in humans is yet known for the new substances tested.

For practical reasons, thawed primary GBM cells were tested although in vitro testing directly after establishing the primary cell culture might prevent potential freezing- and rethawing-induced changes of cell characteristics. In the majority of studies testing tumour cells for cell death induction in vitro, the clonogenic assay or the metabolic MTT assay was used. In our hands, the latter was not reliable due to high intra- and interassay variances. While cell viability measurement by Trypan blue exclusion was used in the study, similar results

obtained by Propidium iodide staining indicated that GMB cells died by apoptosis.

In line with the observations of others [22] among the established anti-tumour drugs, doxorubicin was most potent and significantly more effective in cell death induction than BCNU, vincristin, and γ-irradiation (Fig. 2). However, doxorubicin was shown to induce the multi-drug-resistance proteins in vivo [3] and BCNU and γ-irradiation, but not doxorubicin were shown to augment patients survival in clinical studies. Lack of doxorubicin-induced cell death in vivo is most probably due to the blood-brain-barrier thus suggesting for doxorubicin application methods bypassing the blood-brain barrier like Rickham-reservoirs helping to direct drugs to the tumour site. Whether BA, ceramide, and TRAIL can be applied systemically or would also need local application methods to increase their bio-availability has yet to be tested.

In various, but not all tumour cells, the CD95 system mediates cell death of cytotoxic drugs like doxorubicin. As cell death by CD95 correlated to cell death induction by doxorubicin in our samples, our data underline that common mechanisms for apoptosis resistance might exist for both stimuli thus supporting that the CD95 system might mediate doxorubicin-induced cell death in GBM cells. Only few primary samples showed sensitivity for TRAIL-induced cell death. Resistance of the remaining samples might be caused by apoptosis signalling defects present in GBM cells [7].

Since various decades, in vitro testing of primary tumour cells is performed not only to predict effectiveness but also with the aim of predicting clinical outcome and to individualise chemotherapy protocols. Although in the present study, we were not able to find a statistically significant correlation of anti-tumour response of death inducing ligands in vitro and the clinical outcome of the respective patient, we observed a remarkable effectiveness of all three substances with a clear-cut gap to all cytotoxic drugs tested. As GBM is a highly radio- and chemoresistant, still lethal tumour, further studies are needed to validate these promising results in vivo by analysing the therapeutic potential of BA, ceramide and TRAIL for the treatment of GBM including bio-availability, toxicity, and effectiveness in vivo.

Acknowledgement

The authors would like to thank Prof. Unterberg for helpful discussion and Renata Zucic for excellent technical work. This work was supported by the Bettina-Bräu Stiftung.

References

1. Ashkenazi A, Pai RC, Fong S, Leung S, Lawrence DA, Marsters SA, Blackie C, Chang L, McMurtrey AE, Hebert A, DeForge L, Koumenis IL, Lewis D, Harris L, Bussiere J, Koeppen H, Shahrokh Z, Schwall RH (1999) Safety and antitumor activity of recombinant soluble Apo2 ligand. J Clin Invest 4: 155–162
2. Cox RD (1972) Regression models and life-tables. J Royal Stat Soc Series B 34: 187–220
3. Fenner MH, Possinger K (2002) Chemotherapy for breast cancer brain metastases. Onkologie 25: 474–479
4. Fulda S, Friesen C, Los M, Scaffidi C, Mier W, Benedict M, Nunez G, Krammer PH, Peter ME, Debatin KM (1997) Betulinic acid triggers CD95 (APO-1/Fas)- and p53-independent apoptosis via activation of caspases in neuroectodermal tumors. Cancer Res 57: 4956–4964
5. Fulda S, Wick W, Weller M, Debatin KM (2002) Smac agonists sensitize for Apo2L/TRAIL- or anticancer drug-induced apoptosis and induce regression of malignant glioma in vivo. Nat Med 8: 808–815
6. Herold-Mende C, Steiner HH, Andl T, Riede D, Buttler A, Reisser C, Fusenig NE, Mueller MM (1999) Expression and functional significance of vascular endothelial growth factor receptors in human tumor cells. Lab Invest 79: 1573–1582
7. Jendrossek V, Belka C, Bamberg M (2003) Novel chemotherapeutic agents for the treatment of glioblastoma multiforme. Expert Opin Investig Drugs 12: 1899–1924
8. Jeremias I, Herr I, Boehler T, Debatin KM (1998) TRAIL/Apo-2-Ligand induced apoptosis in T-cells. Eur J Immunol 28: 143–152
9. Kelley SK, Harris L, Xie D, DeForge L, Totpal K, Bussiere J, Fox JA (2001) Preclinical studies to predict the disposition of Apo2L/tumor necrosis factor-related apoptosis-inducing ligand in humans: characterization of in vivo efficacy, pharmacokinetics, and safety. J Pharmacol Exp Ther 299: 31–38
10. Kolesnick R (2002) The therapeutic potential of modulating the ceramide/sphingomyelin pathway. J Clin Invest 110: 3–8
11. Nicoletti I, Migliorati G, Pagliacci MC, Grignani F, Riccardi C (1991) A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. J Immunol Meth 139: 271–279
12. Paul C, Liliemark J, Tidefelt U, Gahrton G, Peterson C (1989) Pharmacokinetics of daunorubicin and doxorubicin in plasma and leukemic cells from patients with acute nonlymphoblastic leukemia. Ther Drug Monit 11: 140–148
13. Rahmani R, Zhou XJ (1993) Pharmacokinetics and metabolism of vinca alkaloids. Cancer Surv 17: 269–281
14. Riva C, Lavieille JP, Schmerber S, Cuisnie O, Reyt E (2000) Phase II trial of cisplatin, 5-fluorouracil and folinic acid using a weekly 24-h infusion schedule for locally advanced head and neck cancer: a pharmacokinetic and clinical survey. Int J Oncol 17: 543–549
15. Roth W, Isenmann S, Naumann U, Kugler S, Bahr M, Dichgans J, Ashkenazi A, Weller M (1999) Locoregional Apo2L/TRAIL eradicates intracranial human malignant glioma xenografts in athymic mice in the absence of neurotoxicity. Biochem Biophys Res Commun 265: 479–483
16. Sakuma S, Sawamura Y, Tada M, Aida T, Abe H, Suzuki K, Taniguchi N (1993) Responses of human glioblastoma cells to human natural tumor necrosis factor-alpha: susceptibility, mechanism of resistance and cytokine production studies. J Neurooncol 15: 197–208
17. Scott JN, Rewcastle NB, Brasher PM, Fulton D, MacKinnon JA, Hamilton M, Cairncross JG, Forsyth P (1999) Which glioblastoma multiforme patient will become a long-term survivor? A population-based study. Ann Neurol 46: 183–188

18. Skalski V, Rivas J, Panasci L, McQuillan A, Feindel W (1988) The cytotoxicity of sarcosinamide chloroethylnitrosourea (SarCNU) and BCNU in primary gliomas and glioma cell lines: analysis of data in reference to theoretical peak plasma concentrations in man. Cancer Chemother Pharmacol 22: 137–140
19. Steiner HH, Herold-Mende C, Bonsanto M, Geletneky K, Kunze S (1998) Zur Prognose von Hirntumoren: Epidermiologie, Überlebenszeit und klinischer Verlauf. Versicherungsmedizin 5: 173–178
20. Walczak H, Miller RE, Ariail K, Gliniak B, Griffith TS, Kubin M, Chin W, Jones J, Woodward A, Le T, Smith C, Smolak P, Goodwin RG, Rauch CT, Schuh JC, Lynch DH (1999) Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand in vivo. Nat Med 5: 157–163
21. Wirtz CR, Knauth M, Staubert A, Bonsanto M, Sartor K, Kunze S, Tronnier V (2000) Clinical evaluation and follow-up results for intraoperative magnetic resonance imaging in Neurosurgery. Neurosurgery 46: 1112–1121
22. Wolff JE, Trilling T, Molenkamp G, Egeler RM, Jurgens H (1999) Chemosensitivity of glioma cells in vitro: a meta analysis. J Cancer Res Clin Oncol 125: 481–486
23. Zuco V, Supino R, Righetti SC, Cleris L, Marchesi E, Gambacorti-Passerini C, Formelli F (2002) Selective cytotoxicity of betulinic acid on tumor cell lines, but not on normal cells. Cancer Lett 175: 17–25

Comment

The effectiveness of chemotherapy as a treatment for glioblastoma multiforme is under continuous debate. One reason for the low response to anti-cancer drugs might be the inherent or acquired multi drug resistance of the tumour, which can differ substantially between the pathologically same tumours taken from taken different patients. Therefore, testing various anti-cancer drugs on primary tumour cell cultures reveals an important picture of the individual tumour characteristics of each patient offering the possibility of an individualized chemotherapy regimen.

Tests with betulinic acid, ceramide and TRAIL have shown promising results in studies on cell death of glioma cell lines and paediatric tumour cultures. The present study on primary glioblastoma samples from adult patients is an important continuation of these studies on the way to a possible clinical application.

Verena Amberger-Murphy
Dublin

Correspondence: Irmela Jeremias, Dr. von Haunersches Kinderspital, Lindwurmstr. 4, 80337 München, Germany. e-mail: I.Jeremias@lrz.uni-muenchen.de