were performed within 14 days, $p < 0.05$). Volume reduction was dependent of both initial volume and necrosis. Statistical modelling of these data after exclusion of lesions with short follow-up resulted in a group with a median initial volume of 5.3 ccm and a median maximum reduction of 3.1 ccm. Thus, each 3 Gy-fraction thus induces a volume reduction of 0.31 ccm. To make a lesion of 5.3 ccm (corresponds to a diameter of 2.15 cm) disappear on CT scans, an equivalent dose of 56 Gy in 2 Gy-fractions is needed.
Conclusions: Because permanent local control requires sterilisation of all clonogen tumor cells incl. those no longer visible on CT after macroscopic CR, even small lesions of just over 2 cm diameter should receive more than 28 fractions of 2 Gy. If 10×3 Gy is to be given together with a sensitising agent and the aim of inducing a CR, the agent must induce killing of the remaining $0.53-0.31 = 0.22$ cm of cells, which means increasing the effect of WBRT alone by 71%.

505 POSTER

Treatment of newly diagnosed high-grade glioma with concomitant and adjuvant temozolomide and radiotherapy – UK experience

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Background: In the UK the current management of high-grade gliomas is maximal surgical debulking followed by radiotherapy. It has been shown that the addition of temozolomide (TMZ) to radiotherapy significantly increases median survival (14.6 vs 12.1 months (Stupp et al, 2005 N Engl J Med 352 (10): 1036–8). Our centre has considerable experience with TMZ and has treated patients with a similar regimen. This study aims to confirm whether these results are replicated in practice in the UK.
Material and methods: We retrospectively reviewed 102 patients treated for high-grade gliomas with radiotherapy ± TMZ between 1998 and 2003. A search of our radiotherapy database and patient records was undertaken. Patients who were diagnosed with high-grade glioma and who did not receive radiotherapy or who only received a palliative dose were excluded from the study. Radiotherapy was administered to a dose of 60–65 Gy in 30–37 fractions over 6 weeks. TMZ was administered orally at a dose of 75 mg/m^2 daily for 6 weeks durig radiotherapy, followed by adjuvant TMZ for 6 cycles on days 1–5 of a 28-day cycle (150–200 mg/m^2/day).
Results: 102 patients (71 male, 31 female) with high grade gliomas were planned for treatment with radical radiotherapy (mean age = 52.6 years, range 21–72). 84 patients had glioblastoma multiforme (GBM), 18 had WHO grade III tumours. 53 patients underwent surgical debulking. 51 patients (50%) received concurrent TMZ and radiotherapy followed by adjuvant TMZ (median number of cycles = 3). 48 patients (47%) initially received radiotherapy alone but 10 of these received chemotherapy on disease progression. 3 patients died before treatment started. The choice of treatment options was partly historical (availability of TMZ) and partly the preference of the treating consultant. There were no identifiable patient factors influencing the decision for radiotherapy alone or combined treatment. Only 2 cases had grade III/IV haematological toxicity during concurrent treatment. Patients treated with concurrent TMZ and radiotherapy had a significantly better median survival by log-rank comparison of 12.5 months compared with 9 months for those treated with just radiotherapy ($p = 0.029$).
Conclusion: The addition of TMZ to the standard treatment of radiotherapy for high grade gliomas gives improved overall survival. This study shows that the published Phase III results can be replicated in everyday practice and that the regimen is both practical and effective.

506 POSTER

Shiga-like toxin inhibits cell viability and induce apoptosis in human glioma cells

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Tumour growth is due to an imbalance between cell proliferation and cell death. Increasing apoptosis (programmed cell death) is the most important mechanism for tumour cell death and tumour mass reduction during treatment with cytostatic drugs and irradiation. We therefore aimed at identifying mechanisms for induction of apoptosis by shiga-like toxin and to study its potential use for increasing the efficacy of tumour treatment. Shiga-like toxins have low adverse effects and are only cytotoxic to eukaryotic cells that express its cell surface receptor CD77. CD77 is over-expressed by several solid tumours such as breast carcinoma, ovarian carcinoma, and brain tumours.
We found that two glioma cell lines (U343 and SF767) that over-expressed CD77 also were sensitive for 0.001–5 ng/mL shiga-like toxin in a fluorometric cytotoxicity assay after 72 h incubation whereas other non-CD77-expressing cells were not. The cytotoxicity of the toxin was due to apoptosis as demonstrated by TUNEL staining after 48 h incubation using flow cytometry. 2 μmol/L of the CD77-receptor analogue PPMP (1-phenyl-2-hexadecanoyl-3-morpholino-1-propanol) eradicated CD77 expression after 3 and 6 days incubation and also completely inhibited shiga-like toxin cytotoxicity and apoptosis in both cell lines.
Our results suggest that shiga-like toxin may be used as a potent cytotoxic drug in the treatment of CD77-overexpressing tumours.

507 POSTER

A role of herpesviruses in brain tumor development?

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Background: Malignant gliomas are the most common primary brain tumors in humans and are generally rapidly fatal despite current therapies. Except for hereditary predisposition and high dose ionizing radiation, risk factors such as occupational, environmental or medical factors are controversially discussed. In addition, a role of viruses is suspected. Recently, Cobbs et al. (Cancer Res 2002, 62:3347–50) reported that of 27 gliomas tested, all expressed multiple gene products of HCMV in contrast to brain tissues from patients with meningioma, stroke, Alzheimer's and other brain diseases suggesting that HCMV might play an active role in glioma pathogenesis. Earlier sero-epidemiological case-control studies reported an inverse correlation of glioma cases with serum antibodies against varicella-zoster virus (VZV), Epstein-Barr virus (EBV) and herpes simplex virus (HSV). HCMV antibodies were slightly more frequently observed in glioma cases than in population controls (Wrensch et al., Am J Epidemiol 2001, 154: 161–5). The present study was conducted to evaluate the role of previous herpesvirus infections in brain tumor development by (i) assessing the prevalence of HCMV gene products and/or nucleic acids in primary brain tumor tissues and corresponding blood samples, and also (ii) analyzing the sero-prevalence of anti-HCMV, anti-VZV, anti-EBV and anti-HSV IgM and IgG antibodies in patients with primary brain tumors.
Material and methods: Of 95 patients with primary brain tumors (gliomas, meningiomas, acoustic neurinomas, and medulloblastomas) biopsies from tumor tissues and blood samples were analyzed by a variety of nested PCRs for the presence of HCMV DNA, and sections of tumor tissues were analyzed by immunohistochemistry to detect HCMV-specific proteins. Furthermore, patients' sera were tested by ELISA for IgG and IgM antibodies to HCMV, VZV, EBV, and HSV, and compared to published prevalences.
Results: HCMV DNA was not detected, neither in the brain tumor tissues nor in the corresponding blood samples. Similarly, immunohistochemistry did not reveal any HCMV-specific proteins. Patients' sera were all were negative for IgM antibodies against the herpesviruses. IgG seroprevalences did not differ from published reference data in the German population.
Conclusion: The present study could not confirm the hypothesis that HCMV or other herpesviruses may play a role in glioma pathogenesis.

508 POSTER

Dexamethasone inhibit anti-cancer agent/radiation-induced apoptosis in C6 glioma cells

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Background: Dexamethasone, a synthetic glucocorticoid, is reported to induce partial resistance to anticancer drugs in glioma cells by transcriptional activation of Bcl-xL gene. In the present study, we investigated the upstream regulator of Bcl-xL gene which is activated by dexamethasone. And the effect of dexamethasone on radiation was also evaluated.
Methods and materials: For the induction of apoptosis in C6 glioma cells, 2 μM of camptothecin was added to the culture medium and up to 10 Gy of radiation irradiated onto cells. Western blot were performed to evaluate the effects of dexamethasone on Bcl-xL. Electrophoretic Mobility Shift Assay (EMSA) was conducted to assess DNA-binding activity of Stat5. To identify physical interaction between Stat5 and glucocorticoid receptor, Co-immunoprecipitation was performed. Cell viability was quantified by clonogenic assay and MTT assay. Apoptotic cell death was confirmed by a colorimetric caspase-3 assay with CaspACE™ (Promega), and DNA breakage by Cell death detection ELISA kit(Roche) or DAPI staining.
Results: Camptothecin alone increased caspase-3 activity up to 15.9 pmol pNA/μg/hour, in contrast to 3.5 pNA/μg/hour in untreated control cells. Increased caspase-3 activity by camptothecin was not seen in cells

pre-treated with 1 μM of dexamethasone. This effect was reversed by RU486 (Glucocorticcoid receptor(GR) antagonist). Upon dexamethasone treatment, phosphorylated Stat5 increased within 2 hr and gradually decreased from 4–6 hr on western blot. On EMSA to investigate nuclear DNA binding activity of Stat5 protein, the binding activity increased gradually up to 4 hour and then decreased thereafter. Nuclear extract was immunoprecipitated with a GR receptor specific antiserum, and developed on immunoblot with a Stat5 specific antiserum. Untreated control cells showed minimal activity of phosphorylated Stat5, whereas cells treated with dexamethasone for 2–4 h had increased phosphorylated Stat5 activity.
Conclusions: Stat5 is activated by dexamethasone treatment in C6 glioma cells, resulting in elevation of Bcl-xL expression and inhibition of camptothecin and radiation-induced apoptosis. Using coimmunoprecipitation, we found that GR binds to phosphorylated Stat5 after dexamethasone treatment.

509 POSTER

In vitro evaluation of glioma cell lines and primary glioma cell cultures chemosensitivities: the effect of pharmacological modulation of peripheral benzodiazepine receptors

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Malignant gliomas are generally known to be highly resistant against anticancer chemotherapy. Besides several different mechanisms of resistance it assumes also the incapability of glioma cells to enter in chemotherapeutic drug-induced apoptosis. An intervention in proapoptotic events is one of the possibilities to influence it. Mitochondrial permeability transition pore (MPTP) represents an important factor in mitochondrial pathway of apoptosis induction. Peripheral benzodiazepine receptors (PBR) form part of MPTP. The aim of the following work was to identify the chemosensitising effect of non-selective PBR ligand (diazepam) on U-87 MG and U-373 MG glioma cell lines and primary cultures of cells isolated from peroperative glioblastoma samples (n = 59).
The chemosensitivity of human glioma cell lines and primary glioma cell cultures was assessed by using colorimetric assay with the MTT endpoint. The cells were cultured with different concentrations of cisplatin (CDDP), etoposide (VP-16) or lomustine (CCNU) alone or in combination with diazepam (10−4 M) for 72 hours. The presence of apoptosis, cell cycle changes and disruption of mitochondrial membrane potential were detected by flow cytometry.
The results indicated that diazepam exerted significant antiproliferative activity in U-87MG cells and primary glioma cells but not in U-373MG cell line. In the same time diazepam enhanced chemosenzitivity to CDDP, VP-16 and CCNU in above mentioned cells except U-373MG. Mechanism of the effect of diazepam resulted from facilitation of chemotherapy-induced apoptosis as shown by increased sub-G0/G1 fraction of cells, higher amount of cells with reduced mitochondrial membrane potential and externalised phosphatidylserine. It was concluded that diazepam as non-selective PBR ligand exerted antiproliferative, chemosensitizing and proapoptotic effect in U-87MG cell line and primary glioma cell cultures. However, it was not effective in U-373MG glioma cell line.
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510 POSTER

The role of HIF-1 alpha; and iNOS in primary brain tumors

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Background: Hypoxia-inducible-factor-1 (HIF-1) is present at high levels in human tumors and plays crucial roles in tumor promotion by up regulating several target genes. HIF-1 stimulates the production of NO through the induction of inducible NO synthase (iNOS). Immunohistochemical demonstration of the subunit HIF-1α in archival pathology material has recently been shown to be adversely associated with prognosis in several tumors, including oligodendrogliomas. iNOS expression was also increased in oligodendrogliomas.
Material and methods: We examined retrospectively the HIF-1α and iNOS expression in 60 human astrocytomas by immunohistochemical method using formalin-fixed paraffin-embedded material. In 39 cases we correlated the results of immunohistochemistry with the clinical outcome.
Results: The HIF-1α was detected only in astrocytomas grade III and IV. Although, we expected that HIF-1α is detected in the nucleus we also observed die for HIF-1α in the cytoplasm. The iNOS expression was increased in astrocytomas grade I, II and III and was decreased in astrocytomas grade IV. iNOS was localized round the capillary vessels as well. Statistical analysis showed that HIF-1α expression and iNOS expression did not correlate directly with patients' survival.
Conclusions: HIF-1α is expressed only in astrocytomas grade III and IV and does not affect patients' survival. Expression of iNOS is increased in low-grade astrocytomas and there is no relationship between the level of expression and the survival of patients. Based on these data we believe that these two factors merit further investigations in order to understand the biology of these tumours. More data are needed from prospective studies.

511 POSTER

Sensitivity of human glioblastomas to chemotherapy is related to expression of neural differentiation markers as detected by flow cytometry

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Reliable molecular predictive markers have not yet been found that would enable prospective identification of individual glioblastoma multiforme (GBM) patients with highest chance to benefit from chemotherapy (CHT). Here, we demonstrate the value of flow cytometry in immunophenotypic characterisation of GBM tumours with possible impact on individualised CHT.
Expression of selected neural and other markers including A2B5, CD34, CD45, CD56, CD117, CD133, EGFR, GFAP, Her-2/neu, LIFR, nestin, NGFR, Pgp and vimentin was analysed by flow cytometry in tumour specimens obtained from 11 GBM (WHO gr. IV) patients. Sensitivity of tumour cells to a panel of chemotherapeutics including BCNU, CCNU, CDDP, DAU, DTIC, TAX, TOPO, VCR and VP-16 was tested by the MTT assay.
Distinct immuphenotypic and chemosensitivity patterns were found in individual GBMs. All tumours were positive for A2B5, CD56, nestin and vimentin. EGFR, NGFR and Pgp were expressed only in minor cell subpopulations. CD45-positive cells were identified as infiltrating leukocytes. Very weak reactivity was observed for GFAP. CD34, CD117, CD133, Her-2/neu a LIFR were tested negative in all tumours. Upon correlation, high A2B5 expression was associated with resistance to TAX ($p = 0.038$) and DTIC ($p = 0.030$) whereas high CD56 expression correlated with resistance to CDDP ($p = 0.033$) and CCNU ($p = 0.017$). In contrast, tumours devoid of EGFR were TAX-resistant while EGFR-positive tumours were sensitive ($p = 0.048$). Interestingly, resistance to CCNU correlated with resistance to CDDP (Spearman's $R = 0.629$, $p < 0.05$), DTIC ($R = 0.633$, $p < 0.05$) and TAX ($R=0.760$, $p < 0.05$), but not to BCNU.
In conclusion, we suggest that combined use of chemosensitivity testing and flow cytometric analysis could be helpful in selecting the most appropriate chemotherapy for individual GBM patients.
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512 POSTER

All-trans retinoic acid-mediated catalase induction is correlated with antiprolifertive effect and radiosensitivity in rat glioma (36B10) cells

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Current main treatment of malignant brain tumors is the postoperative radiation therapy and 5-year survival rate is still below 5% even if chemotherapy is added. So, development of new treatment method is urgent. With the findings of their ability of differentiation, inhibition or reversion of cellular proliferation and carcinogenesis, retinoids have been tried for the treatment and prevention of multiple cancers. All-*trans*-retinoic acid (ATRA) has antiproliferative effect for some animal and human brain tumor cells, but the result of clinical trials with ATRA is modest.
We had found the increased catalase by ATRA in a rat glioma cell line (36B10). So, we investigated whether the increased catalase has any correlation with antiproliferative effect of ATRA and radiation sensitivity. When 36B10 cells were exposed to 10−50 μM of ATRA for 24 and 48 h, the expression of catalase mRNA, protein and activity were increased with increasing concentration and incubation time of ATRA. In 36B10, catalase