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.

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

L. Mirossay[1], M. Sarissky[1], V. Balik[2], P. Mirossay[1], I. Sulla[2], J. Mojzis[1]. *[1]P.J. Safarik University, Faculty of Medicine, Pharmacology, Kosice, Slovak Republic; [2]P.J. Safarik University, Faculty of Medicine, Neurosurgery, Kosice, Slovak Republic*

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

E. Giannopoulou[1], P. Ravazoula[2], A. Antonakopoulou[1], H. Kalofonos[1,2], D. Kardamakis[1,3]. *[1]Lab of Clinical Oncology, Medical School, University of Patras, Patras, Greece; [2]Department of Pathology, Medical School, University of Patras, Patras, Greece; [3]Department of Radiotherapy, Medical School, University of Patras, Patras, Greece*

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.

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

M. Sarissky[1], V. Balik[2], P. Mirossay[1], P. Bohus[3], M. Gajdos[2], I. Sulla[2], L. Mirossay[1]. *[1]Faculty of Medicine, P.J. Safarik University, Department of Pharmacology, Kosice, Slovak Republic; [2]Faculty of Medicine, P.J. Safarik University, Department of Neurosurgery, Kosice, Slovak Republic; [3]Louis Pasteur University Hospital, Department of Pathology, Kosice, Slovak Republic*

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

W. Park[1], H. Jeon[1], J. Yu[2]. *[1]Chungbuk National University College of Medicine, Radiation Oncology, Cheongju, Korea; [2]Konkuk University College of Medicine, Parasitology, Chungju, Korea*

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

activity was decreased with increasing the concentration of aminotriazole (ATZ; 0.1, 1, 10 mM) dose-dependently. ROS was increased with ATRA and it was augmented by the combination with radiation. ATZ decreased ROS production and increased cell survival by ATRA alone or ATRA combined with radiation despite the reduction of catalase. The catalase that is induced by ATRA increases ROS production and radiosensitivity, and excess catalase would be one of the mechanisms for antiproliferaive effect of ATRA.
This study shows new mechanism of antiproliferaive effect of ATRA and will give a basis for cancer treatment in using ATRA alone or combined with radiation therapy through the elucidation of the role of antioxidant enzymes.

513 POSTER

Erythropoietin in patients with malignant extradural spinal cord compression: functional and pharmacokinetic outcomes

D.A. Loblaw[1], L. Holden[1], A. Xenocostas[2], E. Chen[3], S. Chander[1], P. Cooper[4], P.C. Chan[5], C.S. Wong[1]. [1]*Sunnybrook & Women's College Health Science Centre, Department of Radiation Oncology, Toronto, Canada;* [2]*London Health Science Center, Division of Hematology, London, Canada;* [3]*Princess Margaret Hospital/University Health Network, Department of Medical Oncology, Toronto, Canada;* [4]*Sunnybrook & Women's College Health Science Centre, Department of Medical Imaging, Toronto, Canada;* [5]*Sunnybrook & Women's College Health Science Centre, Department of Biochemistry, Toronto, Canada*

Background: Erythropoietin has shown neuroprotectant properties in preclinical and randomized studies. There have been no studies showing that erythropoietin enters the central nervous system in patients with extracranial disease.
Methods: Ten paraparetic patients with malignant extradural spinal cord compression who were eligible for radiotherapy, lumbar puncture and intravenous epoetin alfa were enrolled. Patients received epoetin alfa 1500 U/kg intravenously over 30 minutes followed by a standardized dexamethasone and radiotherapy protocol. A lumbar puncture and venipuncture were performed 24–30 hour post-epoetin alfa infusion. Patients were followed daily during radiotherapy, at weeks 2, 3, 4, 8, 12 and at months 6, 9 and 12.
Results: There were no apparent acute toxicities from the epoetin alfa infusion. Erythropoietin was detectable (range 17–214 mIU/ml) in the cerebrospinal fluid in all 8 patients sampled. Before treatment, 8 patients were non-ambulatory and 2 patients were weak but ambulatory. After treatment, 6 (75%) and 2 (100%) recovered or maintained ambulation and improved at least one functional class after a median time of 15 and 18 days, respectively. Five of seven patients with objective sensory deficits and one of seven catheter-dependent patients recovered. Fifty-five percent had a complete pain response and 22% had a partial response. Eight patients have died with a median survival of 1.5 months.
Conclusions: After an intravenous infusion of epoetin alfa, radiotherapy and steroids, high concentrations of erythropoietin were detectable in the cerebrospinal fluid. Patients with malignant extradural spinal cord compression demonstrated encouraging improvements in neurologic function and pain.

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Combined treatment of experimental gliomas with radiotherapy, radiosensitizing and chemosensitizing gene therapy

K. Lumniczky[1], T. Szatmari[1], G. Huszty[1], S. Desaknai[1], M. Sasvari-Szekely[2], M. Staub[2], G. Safrany[1]. [1]*NCPH – National Research Institute for Radiobiology, Molecular and Tumor Radiobiology, Budapest, Hungary;* [2]*Semmelweis University, Institute of Medical Chemistry, Molecular Biology and Pathobiochemistry, Budapest, Hungary*

Background: The aim of this work was to improve the chemotherapeutic and radiosensitising effects of gemcitabine. Our hypothesis was that increasing the deoxycytidine kinase (dCK) enzyme level that activates gemcitabine within the cells, will lead to increased gemcitabine effects, which could improve the efficacy of chemo- and radiotherapy.
Material and methods: Murine Gl261, rat C6 and 9L and human U373 glioma models were used. The dCK gene was cloned into an adenoviral vector (Ad-dCK). For in vitro proliferation assay cells were transduced with Ad-dCK, treated with Gemcitabine and irradiated. Subcutaneous Gl261 tumors were established in C57BL/6 mice using either wild type or Ad-dCK infected tumor cells. Tumor bearing mice were treated with intraperitoneal injection of Gemcitabine and local tumor irradiation. Tumor growth and survival were followed.
Results: Strong differences were seen in the basal dCK activities of the different glioma cell lines: the murine Gl261 cells showed ten fold higher enzyme activities, than the human and rat glioma cell lines. Intracellular dCK activity was raised by infecting the cells with increasing multiplicities of infection (MOI) of Ad-dCK. Ad-dCK at high MOI was very toxic for Gl261 cells, but did not affect the viability of the other glioma cell lines. The in vitro data showed that increased dCK enzyme activities could not further increase gemcitabine toxicity in Gl261 cells, but gemcitabine itself had a minor radiosensitizing effect. On the contrary, in rat C6 and 9L glioma cells elevated dCK levels could substantially improve both gemcitabine toxicity and the radiosensitising effect. In the case of Gl261 cells, in vivo data are in concordance with the in vitro data: although the combined effect of gemcitabine and radiotherapy has a pronounced synergistic effect (60% tumor free animals after 100 days) compared to mono-therapies (no tumor free animals), increasing dCK levels in the tumor cells did not affect tumor growth or survival. Experiments with C6 and 9L rat models are undergoing.
Conclusions: In the Gl261 model increasing intracellular dCK levels could not improve the chemo- or radio-sensitizing effect of gemcitabine. In the C6 and 9L models elevated dCK levels could increase both the chemo- and radio-sensitizing effect of gemcitabine.

515 POSTER

Potential interest in integrating functional MRI (f MRI) in high-precision RT planning for WHO grade 2 unfavorable and grade 3 supra-tentorial gliomas: first experience with 10 consecutive patients

F. Dhermain[1], D. Ducreux[2], A. Beaudre[1], F. Bidault[3], F. Parker[4], B. Pehlivan[6], C. Haie-Meder[1], D. Lefkopoulos[1], J. Bourhis[1]. [1]*Institut Gustave Roussy, Department of Radiotherapy and Physics, Villejuif, France;* [2]*CHU Kremlin Bicetre, Neuroradiology Department, Kremlin Bicêtre, France;* [3]*Institut Gustave Roussy, Department of Radiology, Villejuif, France;* [4]*CHU Kremlin Bicetre, Neurosurgery Department, Kremlin Bicetre, France*

Introduction: Among new imaging techniques potentially useful in radiotherapy (RT) of patients (pts) with brain gliomas, fMRI is supposed to add informations to conventional MRI (cMRI). fMRI could modify Gross Tumor Volume (GTV) delineation, visualize inside a low-grade glioma small focuses of higher activity (for an RT boost) and identify sites essential for memory and language, to be eventually avoided, these functions being potentially altered by RT. Here, fMRI was evaluated for adults with gliomas of intermediate prognosis, in addition with computed tomography (CT) scan and cMRI, routinely used for RT planning (RTP). The main goal was to evaluate if fMRI could modify CT/cMRI-based RTP.
Description: After biopsy/surgery, 10 adult pts with gr. 2 unfavorable or gr. 3 supra-tentorial glioma were entered in the study. CT scan and cMRI (T1 Gado, T2-weighted sequences) were performed in RT position. fMRI was subsequently performed in the same position using 1) a diffusion tensor imaging (DTI)-based fiber tracking technique, visualizing major white matter tracts, 2) a perfusion-weighted imaging identifying higher perfused areas, 3) cortical activation with memory and language paradigms.
Firstly, only CT scan and cMRI were used for RTP, contouring of GTV was based on T1 Gado for gr. 3 and T2 for gr. 2 gliomas, organs at risks (OaR) were delineated. Then, RTP was made, optimizing GTV coverage and mimimizing irradiation of OaR. Pts were treated according to the conventional RTP and baseline neurocognitive functions were evaluated before RT, then bi-annually.
Secondly, fMRI images were analysed and used to define a "functional" GTV for comparison with the conventional one.
Results: 10 adult pts (mean age of 42 yrs) were included in 6 months, all with an oligodendroglioma component. First symptom was epileptic seizure in 8 pts. In 6 pts, glioma was located in the left-temporal area with a mean size of 6 cm in T2-cMRI, 6 showed a mild signal enhancement. In 7 pts, highly active focuses were identified, within homogenous T2 hypersignal areas. In 5 patients, DTI fiber-tracking showed warped white matter fibers, strongly suggesting brain infiltration beyond cMRI images. Overall, the balistic of RT could be potentially modified in 4 pts.
Conclusion: The preliminary results of this study strongly suggest that the entire spectrum of fMRI can play a major contribution to improve the accuracy of high precision RT in adult pts with non-glioblastoma gliomas.

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p53 and RB suppressor pathways deregulation by HDM2 overexpression in human meningeal hemangiopericytomas. double immunofluorescence and laser scanning confocal microscopy study.

J.C. Martinez[1], J.C. Palomino[2], R. Samaniego[3], A. Cabello[4], J.R. Ricoy[4]. [1]*Instituto Oftálmico, A Patologica, Madrid, Spain;* [2]*University Hospital "12 de Octubre", Neurosurgery, Madrid, Spain;* [3]*University Hospital "G Marañon", Confocal Microscopy Unit, Madrid, Spain;* [4]*University Hospital "12 de Octubre", Pathology (Neuropathology), Madrid, Spain*

Meningeal hemangiopericytomas (MHPC's) are slow growing tumors, that in spite of complete surgical removal followed by radiation, recur and