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

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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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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.

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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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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