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[A1]

Use of nanofibre net for prevention of excessive cicatrisation after neurosurgical procedures. Experimental studies

Andrychowski J[1,2], Czernicki Z[1,2], Baniewicz M[3], Kowalczyk T[4], Sulejczak D[5], Kowalewski A[4]

[1]Department of Neurosurgery Medical University of Warsaw, [2]Department of Neurosurgery Mossakowski Medical Research Centre, Polish Academy of Sciences, [3]Department of the Cell Ultrastructure, Mossakowski Medical Research Centre, Polish Academy of Sciences, [4]Institute of Fundamental Technological Research, Polish Academy of Sciences, [5]Department of Experimental Pharmacology, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

Excessive cicatrisation after neurosurgical procedures is adverse event. This process may lead to improper postoperative course and makes reoperations difficult. In procedures with approaches to vertebral canal, the tissue scar adheres to dura mater, penetrates into the canal and can cause narrowing symptoms, can generate neurologic deficits and cause pain. In the literature this phenomenon is described even for 16% of neurosurgical procedures.

Prevention of scar occurrence and spreading is connected with modification of approach endoscopic access, microscopic access or access with use of isolating substances administered intraoperatively (gels, TachoComb (R), autogenous adipose tissue). Authors have not found in the available literature any case of using isolating substances manufactured in nanomaterial technique in neurosurgical procedures.

Aim of the project: The aim of study project using animal model is morphological assessment of cicatrisation process and assessment of excessive cicatrisation prevention by local use of net manufactured by electrospinning process (nanotechnology) via operational access.

Material: Experimental model – a rat. Animal study groups of different follow-up time – 4, 14, 30, 60 days. Additionally control group was formed. During operation, access in thoracic segment of vertebral column is performed. Isolating material is put on place of performed laminectomy – on dura mater, on surface of spinal cord and intramuscularly. The material is analyzed with use of light and electrone microscopy.

Results: Local cicatrisation can be modified by use of nanomaterial. Various parameters of nanonet and consequently limitation of scar spreading can be obtained. The scar can be limited and modified locally.

Conclusions: Initial observations indicate a possibility of effective use of materials obtained in electrospinning process in excessive cicatrisation prevention.

[A2]

Local invasiveness of meningiomas with low histopathological malignancy. Dilemma in surgical treatment

Andrychowski J[1,2], Taraszewska A[3], Czernicki Z[1,2], Zębala M[1], Urbański B[1]

[1]Department of Neurosurgery, Medical University of Warsaw, [2]Department of Neurosurgery and [3]Department of Experimental and Clinical Neuropathology, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

Meningiomas comprise 13-19% of all primary intracranial neoplasms. The basic method of treatment of intracranial meningiomas is surgical resection of the whole tumour and the infiltrated dura. The classic assessment method for surgical procedure is provided by the Simpson rating scale (1957). This study presents the cases of supratentorial meningiomas which constitute dilemmas in the surgical treatment because of incomplete excision and often recurrence due to infiltrative growth of tumour.

We performed a retrospective review of 105 intracranial meningiomas treated surgically between 2008 and 2010 and selected 32 cases (30%) classified histologically as border benign (G I/II) and atypical (G II) meningioma. Common morphological features of

tumours, as observed intraoperatively, were extensive infiltration of the dura and visible, large contact surface of the tumour and the dura. MRI examinations revealed a peritumoural edema and mass effect. Besides a wide infiltration of dura, the tumours in 9 cases demonstrated infiltration of the brain and/or very tight adhesion with arachnoid and pia mater, resulting in a considerable operative difficulty, especially near functionally important areas. The observed tumour expansion features made it impossible to decide on complete removal. Tumour removal was limited to those parts that were directly responsible for the progression of neurological symptoms. The opening was made in order to reveal as much as possible infiltration of the dura. During the removal of the tumour tissue and the surrounding dura it was observed, however, that the dura might have been further infiltrated, which in the decision-making process translated into the impossibility of complete removal.

Conclusions: The meningiomas of low histological malignancy (G I/II and G II), exhibiting infiltrating, diffuse expansion, are subject to individual surgical decisions, however, due to the nature of the process, radical treatment is impossible. The impossibility of complete removal limits surgical treatment to removing the tumour from functionally important regions.

[A3]

Caspase-3 activation in physiological and pathological aging process of mouse brain

Dorszewska J[1], Karolczak D[2], Różycka A[3], Półtrolniczak A[1], Bugaj R[1], Dezor M[1], Marszałek A[2,4], Jagodziński PP[3], Florczak J[5]

[1]Laboratory of Neurobiology, Department of Neurology, [2]Electron Microscopy Laboratory of Department of Clinical Pathomorphology, [3]Department of Biochemistry and Molecular Biology, Poznan University of Medical Sciences, Poznan, [4]Departament of Clinical Pathomorphology, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, [5]Chair and Department of Neurology, Poznan University of Medical Sciences, Poznan, Poland

As documented by the literature, progressing age and neurodegenerative diseases are accompanied by decreasing numbers of neurons in cerebral cortex and cerebellum and by a decrease in number of synapses.

Alzheimer's disease (AD) is one of the most important neurodegenerative diseases, which number cases increased with age. There are a number of molecular factors involved in the pathogenesis of AD, i.e. amyloid precursor protein (APP) gene. Mutations in the *APP* gene account of familial AD (FAD) causes, by changes in the processing of this gene resulting in increase of β-amyloid (Aβ) level. In double transgenic mouse model of AD mutation of APP695 is associated with early-onset FAD. Aged-transgenic mice elevated level of Aβ which may cause to generate of free radicals. Metabolic disturbances in the aging brain may generate of free radicals as well. Free radicals may oxidize macromolecules in cells, such as DNA. DNA damage may induce apoptosis in wild-type p53-expressing cell. Bcl-2 is an anti-apoptotic protein, which prevents caspase-3 activation. TNF-alpha (tumour necrosis factor) may activate caspase-3. TNF-alpha and Bcl-2 family gene expression regulation is not completely understood in physiological and pathological aging process.

The aim of the study was to analyze of Bcl-2 and Bax, and TNF-alpha proteins, and active caspase-3 levels in the brain of double transgenic mouse model of AD and in age-matched and young controls.

The studies were performed on female mice 6.0-8.0-months-old transgenic (B6.Cg-Tg(APP695)3DBo Tg(PSEN1dE9)S9Dbo/J stain, Jackson Lab., USA), and 8.0-12.0-weeks-old, and 6.0-8.0-months-old controls (C57BL/6J stain). Brains of animals were divided into three structures: cerebral grey matter (GM), subcortical white matter (WM) and cerebellum (C). The level of Bcl-2, Bax proteins was determined with Western Blot method, and active caspase-3 was determined with immunohistochemistry technique.

Our study shown, that the level of Bax and Bcl-2 proteins was higher in all analyzed structures of the brain of transgenic mice, in GM (Bax and Bcl-2, $p < 0.05$ as compared to young controls; Bax, $p < 0.05$ and Bcl-2, $p < 0.01$ as compared to age-matched controls), WM (Bax, $p < 0.01$ and Bcl-2, $p < 0.05$ as compared to young controls) and C (Bcl-2, $p < 0.05$ as compared to age-matched controls) as compared to controls. Moreover, Bax : Bcl-2 ratio was insignificantly lower in all structure of the transgenic mice brain as compared to young and old controls, but in all animals indicated higher level of Bax protein. However, the level of TNF-alpha protein was similar in all analyzed regions of young and old animal brain. In all analyzed structures of the mice brain appear cells with active caspase-3,

but the higher number of these cells occurred in GM of transgenic mice.

It seems that Bcl-2 family proteins are involved in the apoptotic neuronal death in the mouse model of AD by caspase-3 activation.

[A4]

1-methyl-1,2,3,4,-tetrahydroisochinoline is neuroprotective in brain ischemia: *in vitro* and *in vivo* studies

Duszczyk M[1], Kuszczyk M[1], Makarewicz D[1], Salińska E[1], Antkiewicz-Michaluk L[2], Łazarewicz JW[1]

[1]Department of Neurochemistry, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, [2]Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland

1-methyl-1,2,3,4-tetrahydroisochinoline (1MeTIQ) is known for its mild neuroprotective potential of the unclear mechanism, whereas other endogenous tetrahydroisochinoline derivatives present in the mammalian brain have been considered as neurotoxic substances. It was shown that 1MeTIQ exhibits antidopaminergic activity and reduces the neurotoxic effects of MPTP and rotenone, decreasing also the behavioral effects of MK-801 in rats *in vivo*. The results of our previous study demonstrated that 1MeTIQ *in vitro* prevents glutamate-induced excitotoxicity in cultured neurons suggesting that this effect may be ascribed to its inhibitory effect on NMDA receptors. Antagonists of NMDA receptors are known to provide neuroprotection in brain ischemia. Therefore the aim of our present study was to verify *in vitro* putative antagonistic effects of 1MeTIQ on different ligand binding sites of the NMDA receptors and to evaluate its neuroprotective potential in the animal models of brain ischemia. The receptor binding experiments using membranous fractions isolated from the rat brain cortex demonstrated that 1MeTIQ in high micro molar concentrations inhibits in a concentration-dependent manner the specific binding of [^{3}H]D-serine and [^{3}H]MK-801, while the binding of [^{3}H]glutamate remains unaffected. These data indicate that 1MeTIQ may be attributed to NMDA receptor antagonists acting at the glycine binding site. The NMDA receptor antagonism of 1MeTIQ was also demonstrated in experiments utilizing primary cultures of rat cerebellar granule cells submitted to acute NMDA and glutamate excitotoxicity. Under these conditions 1MeTIQ applied at high micro molar concentrations provided a pronounced neuroprotection and significantly inhibited generation of the calcium signal. The *in vivo* ischemic experiments demonstrated that application of 1MeTIQ in the dose of 50 mg/kg 30 min before the insult in the model of global forebrain ischemia in Mongolian gerbils or its repeated application in the same dose after hypoxia-ischemia in the rat model of perinatal asphyxia provided significant neuroprotection. In the gerbils treated with 1MeTIQ we observed the morphological and behavioural symptoms of neuroprotection and the postischaemic hypothermia characteristic for medication of brain ischemia with the NMDA receptor antagonists. Collectively these data show that 1MeTIQ acts is a weak antagonist of the glycine binding site of NMDA receptors, providing the neuroprotection under various excitotoxic and ischemic conditions both *in vitro* and *in vivo*.

[A5]

Different approach to rehabilitation of children with intracranial tumours with regards to its location and grade

Fąfara-Leś A[1], Klasa Ł[1], Adamek D[2], Kwiatkowski S[1], Maryńczak L[1], Kawecki Z[1]

[1]Department of Neurosurgery, University Children's Hospital, Medical College, Jagiellonian University, Cracow, [2]Department of Neuropathology, Chair of Pathomorphology, Medical College, Jagiellonian University, Cracow, Poland

Brain tumours together with acquired brain injury are among the major causes of death and disability. In the paediatric population central nervous system tumours are the most common solid tumours.

Individuals with a brain tumours experience the trauma and uncertain prognosis associated with a grade of tumour, and its direct neurological effects. Also the treatment itself (surgical and oncological) effects physical, cognitive and social functioning.

Nowadays, together with increasingly higher chance of survival, the question of quality of life of CNS tumour patients (and survivors after the successful treatment) and the issue of theirs rehabilitation has become very important.

The first purpose of this study is to underline the importance of rehabilitation in children with brain tumours. Second purpose, on the basis of a few cases of children with brain tumours, is to demonstrate different rehabilitation programmes with regards to tumour location and grade.

To demonstrate this, we choose 6 patients with CNS tumours located both supra- or infratentorially, comparing low grade tumours with high grade ones. Depending on histological examination various rehabilitation programmes were performed with different results.

Children with brain tumours require physical treatment according to primary damage by the tumour and secondly, because of the impact of the iatrogenic effects of surgical treatment.

Different rehabilitation projects are necessary for children and adolescents living after brain tumours.

Concerning surgical and oncological treatment, and the grade of tumour it is very important to define adequate programme of rehabilitation.

The physiotherapist dealing with children with brain tumours should be fully aware of the impact and meaning of the neuropathological diagnosis and, as much as possible, should predict the effects of the tumour as well as the surgical and adjuvant treatment on the physical and cognitive state of the patient, adapting correspondingly the approach to rehabilitation.

[A6]

COMT gene polymorphism and parameters of blood pressure in patients with Parkinson's disease

Florczak J[1], Dorszewska J[2], Różycka A[3], Półrolniczak A[2], Bugaj R[2], Wolny Ł[2], Owecki M[1], Jagodziński PP[3], Kozubski W[1]

[1]Chair and Department of Neurology, [2]Laboratory of Neurobiology, Department of Neurology, [3]Department of Biochemistry and Molecular Biology, Poznan University of Medical Sciences, Poznan, Poland

COMT (catechol-O-methyltransferase) gene polymorphism plays an important role in pathogenesis of many neurological diseases including Parkinson's disease (PD), and may be involved in regulation of catecholamine (norepinephrine, NE; epinephrine, E) levels. Catecholamines are important neurotransmitters in the mammalian brain and peripheral sympathoadrenal medullary. Measurement of catecholamines concentrations provide significant information about regarding sympathoadrenal activity. NE and E participate in blood pressure regulation in the autonomic system. Many patients with PD show symptoms of autonomic dysfunction.

The aim of the study was to estimate the frequency of *COMT* (c.649G>A) gene polymorphism in DNA isolated from whole blood samples and analysis catecholamines concentration (NE, E) in plasma of PD patients and in controls.

The investigated group included 53 patients with PD, 28 females and 25 males, aged 35-82 years (62.6 ± 9.9 years, mean age ± SD) and 48 control subjects, 33 females and 15 males, 25-76 years of age (55.8 ± 10.0 years, mean age ± SD). *COMT* gene polymorphism was determined using PCR-RFLP method and the levels of NE and E were estimated using HPLC/EC technique. Measurement of blood pressure and plasma concentration of NE, E was performed after 30 min. of supine position and then 5 min. of upright position.

Our study indicated an increased number of both GA heterozygotes (c.649G>A *COMT*) and homozygotes for the incorrect AA genotype (c.649G>A *COMT*) in PD patients. In patients being GA heterozygotes (c.649G>A *COMT*) genotype (Wilcoxon test, $p < 0.05$) there was a significant decrease in blood pressure between the supine and upright position (Wilcoxon test, $p < 0.05$). In PD patients with the wildtype homozygous GG (c.649G>A *COMT*) genotype (Wilcoxon test, $p < 0.05$) and homozygotes for the incorrect AA genotype (c.649G>A *COMT*) (Wilcoxon test, $p < 0.05$) there was a significant increase in NA concentration between the supine and upright positions. Moreover, in PD patients and control subjects being GA heterozygotes (c.649G>A *COMT*) we found a decrease in NA concentration between the supine and upright positions that was significant only in controls.

It seems likely that *COMT* (c.649G>A) gene polymorphism may affect blood pressure and NE levels in patients with PD and in control subjects.

[A7]

Nanotechnology in diagnostic and pharmacotherapy – future medicine

Frontczak-Baniewicz M[1], Kowalewski TA[2]

[1]Department of Cell Ultrastructure, Mossakowski Medical Research Centre, Polish Academy of Sciences, [2]Department of Mechanics and Physics of Fluids, Institute of Fundamental Technological Research, Polish Academy of Sciences, Warsaw, Poland

Nanotechnology is the rapid evolving science of manufacturing and utilizing extremely small particles and devices, sometimes as small as single atoms and molecules. Since its eruption in the late 1980s it already brought a new generation of materials with superior mechanical and electrical properties. The practical applications of nanotechnology can be traced to advances in communications, engineering, physics, chemistry, biology, and robotics.

Nanotechnology is especially important to medicine because the medical field deals with things on the smallest of levels. Biology and medicine, usually employ dispersed nanoparticles, for instance as fluorescent biological labels, drug and gene delivery agents, bio-detection of pathogens, detection of proteins, probing of DNA structure, tissue engineering, tumour destruction via heating (hyperthermia), separation and purification of biological molecules and cells, magnetic resonance imaging (MRI) contrast enhancement. Additionally, the small nano devices that are being developed right now can enter the body and look around in ways that large humans can only dream of.

We focus on applications in the cellular and intracellular delivery of therapeutic agents and explore various types of nanoparticles and nanofibres. Because of the remarkable drug delivery challenges in the central nervous system's blood-brain barrier, helpful examples of nanoparticles in the treatment of neurological cancer, neurovascular disorders, and neurodegenerative diseases are discussed. Promising results are obtained with especially designed nanofibreous mats applied as neuroprotective surgical dressing.

Medical therapies have become more personalized to specific diseases and most pharmaceutical agents have primary targets within cells and tissues. Moreover, these agents may be preferentially delivered to these sites of action within the cell. Selective subcellular delivery is likely to have greater therapeutic benefits. Cytosolic delivery is desirable for drugs that undergo extensive exportation from the cell via efflux transporters such as multi-drug resistance proteins. These mechanisms continuously reduce therapeutic intracellular drug concentrations. Potential treatments for common neurological disorders, such as stroke, tumours and Alzheimer's, are therefore a much preferred application of nanomedicine.

[A8]

Polymorphism within *APBB2* gene in Polish centenarians

Golanska E[1], Sieruta M[1], Gresner SM[1], Klich I[2], Pfeffer-Baczuk A[3], Szybinska A[4], Sobow TM[5], Liberski PP[1]

[1]Department of Molecular Pathology and Neuropathology, Medical University of Lodz, [2]Department of Children's Diseases, Medical University of Lodz, [3]Department of Neurodegenerative Disorders, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, [4]International Institute of Molecular and Cell Biology, Warsaw, [5]Department of Medical Psychology, Medical University of Lodz, Lodz, Poland

Human life span is believed to be influenced by environmental as well as genetic factors. Centenarians, attaining unusual age beyond the average life expectancies, comprise a group helpful in those investigations as they may have rare genetic combinations beneficial for long life and/or protective against major age-related disorders. In this study, we searched for a possible association between rs13133980 single nucleotide polymorphism (SNP) within *APBB2* gene with longevity and dementia in a population of Polish centenarians. *APBB2* gene codes for amyloid beta precursor (APP) – binding protein family B member 2, which is expressed in brain regions important for learning and memory. It was shown that overexpression of *APBB2* increases γ-secretase activity and may generate a main constituent of senile plaques: Aβ peptides. SNPs within *APBB2* gene were proposed as potential risk factors for Alzheimer's disease and to date have not been analyzed in long-living individuals. We found no significant difference between genotypes frequency in centenarians compared to young controls, however, after stratification of centenarians into two groups: with and without dementia, we observed over-representation of G allele in individuals with

dementia. Our preliminary data suggest that the analyzed genetic polymorphism is not associated with longevity, however it may influence cognitive performance in the elderly.

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[A9]

Molecular alterations in meningiomas – preliminary studies

Grešner SM[1], Jaskólski DJ[2], Piaskowski S[1], Stawski R[1], Woźniak K[1], Sikorska B[1], Papierz W[3], Rieske P[1], Liberski PP[1]

[1]Department of Molecular Pathology and Neuropathology, Medical University of Lodz, [2]Department of Neurosurgery, Medical University of Lodz, [3]Department of Pathomorphology, Medical University of Lodz, Lodz, Poland

Meningiomas are the most common benign intracranial tumours in adults arising from the arachnoidal cells. Several risk factors connected with meningioma development have been described. The most important are ionizing radiation, head injury, hormones and genetic factors including the loss of heterozygosity (LOH).

The aim of our study was to evaluate the frequency of deletions on chromosome 14 and correlate them with clinical data. To this end, we reviewed 76 benign (WHO grade I) and 15 atypical (WHO grade II) meningiomas. We found deletions on chromosome 14 to be significantly associated with tumour grade, with LOH frequency in atypical meningiomas (WHO grade II) being higher compared to the one in benign meningiomas (WHO grade I) (30.7% vs. 8.6%; $P = 0.05$). Patients with deletions on chromosome 14 were found to be at approximately 5-fold higher risk for development of atypical instead of benign meningiomas (OR = 4.7; 95% CI: 1.05-20.96). Moreover, we found a significant link between deletions on chromosome 14 and tumour size. Subjects were divided into two groups with respect to the tumour diameter median (M = 4 cm). The LOH frequency was higher in the case of tumour with diameter > 4 cm (21.05% vs. 4.35%; $P = 0.05$).

Our data provide evidence that loss of heterozygosity on chromosome 14 is associated with meningioma development.

[A10]

Malignant transformation of an intracranial epidermoid cyst – case report

Jarosz B[1], Rola R[2], Janczarek M[3], Trojanowski T[2]

[1]Neuropathological Laboratory of the [2]Chair & Department of Neurosurgery and Pediatric Neurosurgery, Medical University of Lublin, [3]Department of Neuroradiology, Medical University of Lublin, Lublin, Poland

Primary intracranial squamous cell carcinoma is extremely rare. Most of the cases arise as a result of malignant transformation of an epidermoid or a dermoid cyst.

A 62-year-old female was treated many years for temporal lobe epilepsy. The frequency of epileptic attack increased and amnesic dysphasia appeared and therefore CT and MRI was made which revealed tumour of the left temporal lobe of the brain with suspicion of glioma.

Left fronto-temporal craniotomy was performed and the tumour was partially removed.

The tumour was situated 15-20 mm under cortex and was grown together with dura mater of the middle cranial fossa. In the middle of the tumour there were two cysts with yellowish masses.

The histological diagnosis was epidermoid cyst with malignant transformation to the squamous cell carcinoma. In the wall of the cyst there is more differentiated part of the squamous cell carcinoma. In the tumour there is also poorly differentiated squamous cell carcinoma. The yellowish masses there are keratotic masses. Tumour cells were immunohistochemically positive for cytokeratin AE1/AE3 and EMA and negative for CK7 and CK20.

[A11]

Atypical teratoid/rhabdoid tumour of the thoracic spine in adult – case report

Jarosz B[1], Osuchowski J[2], Janczarek M[3], Ciechańska M[4], Juszczyńska J[5], Trojanowski T[2]

[1]Neuropathological Laboratory of the [2]Chair & Department of Neurosurgery and Pediatric Neurosurgery, Medical University of Lublin, [3]Department of Neuroradiology, Medical University of Lublin; [4]Department of Chemotherapy and [5]Department of Teleradiotherapy, Lublin Oncology Center, Lublin, Poland

Atypical teratoid/rhabdoid tumour of the central nervous system is a rare mainly paediatric neoplasm with poor prognosis. The first example affecting the CNS was reported in 1985. Spinal location and adult cases are rare.

A 25-year-old female with weakness of the right lower limb and next with paresis of both limbs has been taken to the Neurosurgical Clinic. Magnetic resonance imaging of the spinal cord revealed an intramedullary mass at the level of TH7. The size of the tumour was 30 × 30 × 10 mm. The tumour was surgically removed. Tumour cells were immunohistochemically positive for cytokeratin AE1/AE3, focally for Synaptophysin and negative for S-100, LCA, GFAP, CD99, PLAP. TTF-1 was ambiguous. Labelling index MIB-1 was 32.5%. The diagnosis was *Microcellular carcinoma*. PET of the whole body did not reveal primary tumour. Magnetic resonance imaging of the spinal cord four months after surgical operation revealed recurrence of the tumour. After second operation she was re-diagnosed. Immunohistochemistry was repeated and supplemented – vimentin was positive and PAS in cytoplasm was positive. The final diagnosis is AT/RT. Now the patient is during chemo- and simultaneously radiotherapy of the cerebro-spinal axis.

[A12]

What is the real goal of treatment of DNT?

Klasa Ł[1], Fąfara-Leś A[1], Adamek D[2], Maryńczak L[1], Kwiatkowski S[1], Kawecki Z[1]

[1]Department of Neurosurgery, University Children's Hospital, Medical College, Jagiellonian University, Cracow, [2]Department of Neuropathology, Chair of Pathomorphology, Medical College, Jagiellonian University, Cracow, Poland

Introduction: Dysembryoplastic neuroepithelial tumour DNT occurs mainly in children and young adult population with frequency of 1.5-2.3% with some prevalence of men. DNT has typically intracortical location with nodular structure and is composed of mixed glial and neuronal components. DNT is characterized by low grade of malignancy (WHO grade I) and though being histologically bland, its biological activity manifests first of all by epilepsy. The origin and pathogenesis of this tumour is not obvious, and its position in the "spectrum" between a cortical malformation and a true neoplastic tumour is not fully established, however noteworthy is the fact that in most cases foci of cortical dysplasia are observed. DNT is mostly located in temporal lobe, rarely in frontal, parietal and occipital lobe, sporadically in cerebellum and basal nuclei. Clinically DNT is manifested by drug resistant partial complex epileptic seizures with or without secondary generalization. Other symptoms related to increased intracranial pressure occur rarely, especially in advanced stadium of disease.

The aim of this study: To review the cases of children with DNT operated in the Ward of Neurosurgery of the University Children's Hospital in Cracow with regards especially to the course of the disease and its final outcome.

Material and methods: Retrospective analysis of cases of 6 children aged 3-14 years operated in the years 2004-2011 in the Neurosurgery Ward of the University Children's Hospital in Cracow. In all cases DNT was histologically confirmed. The age of children at time of diagnosis, duration of symptoms (epilepsy) before diagnosis, anticonvulsants pharmacotherapy and applied surgical treatment were analyzed.

Results: We observed partial complex epileptic seizures with tendency toward secondary generalization in almost every patient in the analyzed group. The longest period between first epileptic seizure and diagnosis of DNT was 16 months, and the shortest one –

2 months. In 5/6 patients, after surgical treatment the persistent reduction of epileptic seizures was achieved.

Conclusions: Early institution of surgical treatment with total resection of the tumour in patients with DNT leads to not only improvement quality of life trough reduction of the number and severity of seizures but also prevents the relapse and recurrence of neoplasm. Since the extent (totality) of the resection matters, crucial factor is proper intraoperational neuropathological diagnosis (confirmation) of DNT. Treating DNT, we first of all treat epilepsy but cautious follow-up is mandatory to exclude recurrence.

[A13]

Pathological spectrum of cerebral cortex disturbances in human holoprosencephaly. Disturbances of cortical development in human holoprosencephaly

Laure-Kamionowska M[1], Szymańska K[1], Taraszewska A[1], Ogonowska W[1], Klepacka T[2], Deręgowski K[3]

[1]Department of Clinical and Experimental Neuropathology, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, [2]Department of Pathomorphology, Institute for Mother and Child, Warsaw, Poland, [3]Department of Pathology, Hospital General Lanzarote, Arrecife, Lanzarote, Islas Canarias, Spain

Holoprosencephaly is the malformation of the CNS with undivided prosencephaly into two hemispheres and with undivided ventricle. The failed division of hemispheres and ventricle influences on the paired periventricular structures development, like nests of germinal cells, the origin of cells formatting the cerebral cortex. The disturbances in the source of migrating neurons can be reflected in the formation of cortical mantle. The investigation of the cortical alterations in holoprosencephaly cases was the aim of this presentation. We analyzed the frontal cortex of cases with semilobar holoprosencephaly, aged from 24 till 40 gestational weeks. In all cases the disturbances of cortical layering were observed. The main changes concerned the layer II of the cerebral cortex. In the youngest cases the rounded, irregular aggregations of neurons within the second layer were observed. In the older ones radially arranged clusters of cells, or the paucity of neurons in the second layer were found. The other cortical plate abnormalities found in all cases consisted of gliomesodermal heterotopias to the pia mater – the sequel of glial-pial membrane defect. The neuronal overmigration to the molecular cortical layer formed the glioneuronal heterotopias. In some cases the abnormal gyral patterns like polymicrogyria or complete disorganization of all cortical layers were observed. The cortical alterations in holoprosencephaly cases presented mainly two patterns: defect of layer II arrangement and glioneuronal heterotopias. The disturbances of cortical layers appearance can be related with defects of neuronal migration and pial-glial membrane formation.

[A14]

Morphological picture deposits of granular osmiophilic material (GOM) in CADASIL angiopathy

Lewandowska E[1], Dziewulska D[2,3], Szpak GM[1], Pasennik E[1]

[1]Department of Neuropathology, Institute of Psychiatry and Neurology, [2]Department of Neurology, Medical University of Warsaw, [3]Department of Clinical and Experimental Neuropathology, Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

CADASIL is a systemic vascular disease caused by mutations in NOTCH 3 gene. Mutations in the NOTCH 3 gene lead to the accumulation of abnormal Notch 3 ectodomain ($N3^{ECD}$) on the surface of degenerating vascular smooth muscle cells (VSMC). The presence of granular osmiophilic material (GOM) in the thickening of vessel walls is the main pathological finding in electron microscopy. Although clinical manifestations are only cerebral, morphological changes in vessels, including the presence of GOM, are also observed in other organs, e.g., muscles and skin. GOM deposits were located within the basement membrane nearby VSMC and pericytes, often in cell membrane infoldings. Their origin, chemical nature and function are mysterious. A relationship of GOM deposits also remains unknown. In the opinion of some authors $N3^{ECD}$ is accumulated in close proximity to GOM deposits.

The study was aimed at investigating the morphology of GOM deposits in eight skin and muscle biopsy specimens obtained from CADASIL patients.

Electron microscopy showed that the skin and muscle blood vessels of our patients contain numerous

GOM deposits around degenerating VSMC and pericytes or in their membrane infoldings, as well as in some distance from degenerating cells. A thorough ultrastructural examination revealed different morphology of GOM deposits, including size, shape and osmiophilic density. Some of them exhibited irregular, bizarre shapes and various electron density of granular material. Osmiophilic material of high density was frequently observed in part of GOM deposits located near VSMC or pericyte body while a part localized distally from cell body was less dense and loose. In vessels with prominent degeneration of VSMC or pericytes and thickened basement membrane GOM deposits revealed various electron density, sometimes resembling electron density of basement membrane. In our opinion, progressing with distance from VSMC or pericyte rarefaction of granular material in GOM deposits with accompanying characteristic changes in their shapes suggest gradual breakdown of GOM lodgements.

[A15]

Cytotoxic effect of pentabromobenzylisothioureas analoges on human glial-derived tumour cells

Łazarczyk M[1], Kazimierczuk Z[2], Grzywaczewska E[1], Matyja E[1]

[1]Department of Experimental and Clinical Neuropathology, [2]Department of Experimental Pharmacology, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

The anti-cancer properties of pentabromobenzyloisothioureas were proven in experiments on various types of cell lines including: rat C6 glioma cells, human astrocytoma LN229 cells, human leukaemia HL-60 cells and human chronic erythromyeloblastoid leukaemia K-562 cells. Predominantly the proapoptotic effect of these compounds has been documented in these studies.

We investigated the effect of a number of pentabromobenzyloisothioureas derivatives called ZKKs (ZKK-1, ZKK-2, ZKK-3 and ZKK-10) on proliferative activity of human glioblastoma T98G cell and cell line derived from human subependymal giant cell astrocytoma (SEGA). After 72-hour incubation of tumour cells in medium supplemented with these substances in 1 μ – 10 μM concentrations the decrease of neoplastic cell number of both lines was observed. The most potent antiproliferative effect, using Multisizer3 counting, was obtained with ZKK-2. The antiproliferative activity occurred in dose- and time-dependent manner.

The presented results suggest that novel bromobenzyloisothioureas might be considered as promising agents in anticancer therapy and encourage for further identification of their new analogues.

[A16]

Effect of platinum (II) peptide complexes on human glioblastoma T98G cells

Łazarczyk M[1], Głowińska-Grochowska A[3], Leśniak A[2], Zielińska M[1], Lipkowski WA[2], Misicka-Kesik A[3], Matyja E[1]

[1]Department of Experimental and Clinical Neuropathology, [2]Department of Neuropeptide, Mossakowski Medical Research Centre, Polish Academy of Sciences, [3]Laboratory of Peptides, Department of Chemistry, University of Warsaw, Poland

Platinum compounds such as carboplatin or cisplatin are currently applied in chemotherapy of various types of cancer diseases. An anti-tumour activity of the cytostatics relies on intercalation in DNA that prevents cell divisions and induces apoptosis. Irreversible binding of these drugs to sulfhydryl groups of enzymes and other proteins including gluthatione alters cell metabolism.

We investigated the effect of novel platinum compounds: AG83, AG91, AG99 on malignant glioma cell proliferation. The general structure of the substances consists of an opioid peptide derivative (pharmacophore) being a ligand to opioid receptors and a cisplatin derivative (toxicophore). Previous results obtained from radioligand binding assay demonstrated their high affinity to μ and relative lower affinity to δ opioid receptor type. Earlier data derived from immunocytochemical detection indicated, that T98G cells displayed high immunoexpression of μ opioid receptor type but low immunoexpression of δ opioid receptor.

A three-day exposure of glioma T98G cells to AG83 and AG91 in 5 μM and 20 μM concentrations resulted in tumour cell number decrease in a dose- and time-dependent manner. Cell counting was carried out using Multisizer3 (BC). The potential mechanism of the

observed effect may be explained on the basis of known properties of cisplatin mentioned above.

The obtained results are yet another valuable addition in the rapidly developing field of novel anti-cancer therapies.

[A17]

Neuroprotection with hypoxic postconditioning in the rat model of birth asphyxia: two windows of opportunity

Makarewicz D, Salinska E

Laboratory of Pharmaconeurochemistry, Department of Neurochemistry, Mossakowski Medical Research Centre, PAS, Warsaw, Poland

Lack of progress in clinically- applicable neuroprotective strategies in brain ischemia causes increasing interest in alternative methods of therapy, including induction of brain tolerance by pre- and postconditioning. It is known for a long time that hypoxic preconditioning reduces brain damage in the rat model of perinatal asphyxia (Vannucci *et al*., 1998, Cantagrel *et al*., 2003). There are reports demonstrating that also postconditioning with moderate hypoxia delayed for 1 or even 5 days after focal brain ischemia results in a modest neuroprotection in adult mice (Leconte *et al*., 2009), however such studies using the immature rats were never done. It has been suggested that similar mechanisms are involved in the induction of tolerance to brain ischemia by pre- and postconditioning. Two temporal profiles of brain tolerance induced by preconditioning have been recognized: an early tolerance induced within minutes and depending on fast post-translational modifications of proteins and a delayed one, developing after several hours and lasting days, and depending on *de novo* protein synthesis (Kirino, 2002). It is not clear whether brain tolerance to hypoxia/ischemia induced by hypoxic postconditioning is also a two-phase phenomenon. The aim of this study was to evaluate effectiveness of normo- and hypobaric postconditionig initiated 1, 3, or 6 hours after the insult in 7-day-old rats. Hypoxia-ischemia (H-I) was induced by ipsilateral carotid occlusion followed by 75 min. exposure to hypoxia (7.2-7.4% O_2 in N_2). Hypoxic postconditionig was conducted under normobaric conditions at 10% O_2 in N_2 for 75 min, or in the hypobaric chamber set at 360 torr corresponding to 10% O_2 at the sea level. The postconditioning was repeated once a day for 3 consecutive days. The brain damage was evaluated two weeks after H-I and expressed as ipsilateral hemisphere weight deficit in percent of the contralateral hemisphere. Our results demonstrated that both, normo- and hypobaric hypoxic postconditioning resulted in a significant neuroprotection only if initiated 1 h or 6 h after H-I, but not after 3 h. These results demonstrate for the first time efficacy of hypoxic postconditioning in the rat model of H-I and suggest that depending on time of the hypoxic postconditioning, the early and delayed tolerance may be achieved. Experiments verifying the role of mild oxidative stress in the mechanisms of tolerance induced by hypoxic postconditioning are in progress.

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[A18]

Reactive astrocytes participating in resolution of inflammatory reaction of rat brain injured by perinatal asphyxia

Maślińska D[1,2], Kaliszek-Kiniorska A[2], Toborowicz J[1], Gajewski M[3], Rzodkiewicz P[3], Maśliński S[2,3]

[1]Department of Experimental and Clinical Neuropathology, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, [2]Warsaw Medical University, [3]Institute of Rheumatology, Warsaw, Poland

Astrocytes comprise a heterogenous cell population which plays a complex role in physiology and under various pathological conditions that affect the brain. Environmental cues, associated with the brain development, up regulate in astrocytes expressions of various proteins including the glial fibrillary acidic protein (GFAP) which is the standard marker for "activated" astrocytes. The response of such astrocytes to pathological cues is poorly understood. It has been suggested that an inappropriate reaction of these cells to pathological insults may be a cause of the defective brain healing and formation of brain cavities instead of glial scars. Moreover it is not clear whether GFAP-positive astrocytes located in the brain areas distal to the lesion site, may simultaneously respond to the same pathological insult.

In the present study, we examine the response of astrocytes using the experimental model of the rat brain, injured by the perinatal asphyxia (ischemia/hypoxia). The insult causes a focal necrosis in one hemisphere of the brain while the contralateral hemisphere is uninjured. The group of intact age-matched rats was also used as controls.

To assess the effect of perinatal asphyxia on GFAP expression, we measured the relative GFAP mRNA: immediately, 6 hours, 24 hours, 3 days, 5 days, and 7 days after insults. The results showed that, in the injured hemisphere astrocytes responded to asphyxia by up-regulation of GFAP synthesis within the first 24 hours. Such effect was not observed in the contralateral (uninjured) hemisphere suggesting that GFAP-positive astrocytes located in this brain region did not respond to the insult. We confirmed the above conclusion using immunohistochemical methods and the specific antibodies generated against GFAP antigen. The distribution of GFAP-immunopositive astrogliosis only in injured hemisphere was found but in this brain area, 7 days after asphyxia, progressive cavitation was observed. The process of cavitation was associated with the formation of a dense astrogliotic network bordering the lesions. Astrocytes creating such border expressed metallothioneins, the proteins primarily produced by astrocytes that respond to pathological insults. The metallothionein immunoexpression in the uninjured hemisphere was not found.

All our results lead to conclusions that during postnatal brain development, GFAP-positive astrocytes, located at the lesion site, respond immediately to the pathological insult, but it does not prevent the progressive process of cavitation. The subset of GFAP-immunopositive astrocytes bordering such lesions are different than the subset of GFAP-immunopositive astrocytes located in the uninjured hemisphere. These two subsets of astrocytes can be distinquish from each other by the expression of metallothioneins.

[A19]

Immunodistribution of amyloid beta protein and advanced glycation end product receptors (RAGE) in choroid plexus and ependyma of resuscitated patients

Maślińska D[1,2], Opertowska J[1], Deręgowski K[4], Wąsowska L[1], Maśliński S[2,3]

[1]Department of Experimental and Clinical Neuropathology, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, [2]Warsaw Medical University, [3]Institute of Rheumatology, Warsaw, Poland, [4]Department of Pathology, Hospital General Lanzarote Arrecife, Lanzarote, Islas Canarias, Spain

Glycation is a non-enzymatic process which causes post-translational modifications of various proteins by reducing sugars to form of advanced glycation end-products (AGEs), such as amyloid beta protein (Aβ), tau, transthyretin and other proteins associated with several neurodegenerative diseases. Although brain compartments are effectively isolated from the plasma proteins by the blood-brain barrier (BBB), localized on the endothelium of the brain capillaries, there are specialized receptors at this barrier that may shuttle all these proteins in efflux and influx directions. Efflux transport of AGEs through the BBB is mediated by the endothelial LRP-1 (Lipoprotein Receptor-related Protein-1), whereas influx transport involves RAGE (Receptor for Advanced Glycation End-products). The proper function for both types of receptors at the BBB is critical for the regulation of protein homeostasis in the brain. In aging, dysfunction of BBB and increased plasma levels of AGEs may lead to an accumulation in the brain of neurotoxic proteins such as Aβ. Because little is known of the RAGE operating in brain barriers such as those in the choroid plexus and ependyma, the aim of the present study was to examine the immunodistributions of RAGE and Aβ peptides in the choroid plexus where the blood-cerebrospinal fluid barrier (B-CSF) is located, and in ependyma of the brain ventricles associated with functions of the cerebrospinal fluid-brain barrier (CSF-B).

The study was performed on patients of 65 years or older that were affected by total ischemia caused by cardiac arrest. Thirty of these patients were successfully resuscitated and survived a few weeks before they died and following autopsy their brains were used for the study. The control group consisted of thirty,

age-matched individuals who were not resuscitated and died immediately after cardiac arrest. All brains were fixed in formalin and embedded in paraffin. The antibodies generated against different domains of RAGE and Aβ peptide were purchased from Santa Cruz and Sigma Laboratories and used for immuno-histochemical studies. Distribution of the antigens was examined by light and electron microscopy.

In the choroid plexus (CP) and ependyma of resuscitated patients, we found RAGE receptors precisely localized in cells that form the barriers, but not in controls. Thus, we found RAGE in epithelial cells of CP and in ependyma cells bordering the brain ventricles. No RAGE receptors were detected on the endothelium of blood vessels because in contrast to the brain, CP blood vessel walls are fenestrated and proteins may penetrate CP matrix. The selective transport to the cerebrospinal fluid requires special receptors such as RAGE. Results of our study demonstrated that CP is equipped with such receptors and can participate in the influx of glycated proteins, including Aβ from the blood to the CNS. Moreover, our results suggest that severe ischemia caused by cardiac arrest may stimulate the expression of RAGE in cells creating barriers and may increase the transport of Aβ into the brain. Using electron microscopy we demonstrated also the presence of Aβ within the CP blood vessels and in the basement membrane of the CP epithelium. We found this protein also in numerous cytoplasmic vacuoles of epithelial and ependyma cells and observations suggest that the content of these vacuoles were undergoing progressive digestion. The peripheral part of the vacuole content appeared undigested and formed characteristic rings or scrolls, all being immunopositive with Aβ antibodies. Our findings suggest that epithelial and ependyma cells play not only an important role in the creation of amyloid deposits in the brain, but are also places where Aβ may be utilized.

In conclusion, our results strongly support the observations that the RAGE transportation system should be a main target in the therapy of brain amyloidosis which is a known risk factor for Alzheimer's disease.

[A20]

Pilocytic astrocytoma: spectrum of histopathology and consideration of diagnostic pitfalls

Matyja E[1,2], Grajkowska W[1,2], Kunert P[3], Barszcz S[2], Bonicki W[2], Gębarowska J[1], Roszkowski M[5]

[1]Department of Experimental and Clinical Neuropathology, Mossakowski Medical Research Centre, Polish Academy of Sciences, [2]Department of Neurosurgery, M. Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology, [3]Department of Neurosurgery, Warsaw Medical University, [4]Department of Neurosurgery, Institute of Psychiatry and Neurology, [5]Department of Neurosurgery, The Children's Memorial Health Institute, Warsaw, Poland

Pilocytic astrocytomas (PAs) are slowly growing astroglial tumours corresponding to WHO grade I lesions. They occur most commonly in children and young adults, constituting 10% of cerebral and 85% of cerebellar tumours. Pilocytic astrocytomas are tumours displaying a wide range of histological pattern.

Typically, PA is a well circumscribed and often cystic tumour composed of well recognizable biphasic pattern with eosinophilic granular bodies and Rosenthal fibres. Rosenthal fibres are not diagnostic features of PA and they are common in long-standing piloid gliosis associated with craniopharyngioma, cyst wall of hemangioblastoma, spinal ependymomas or pineal cyst. Similarly, EGBs may serve as markers of slowly-growing, prognostically favourable tumour as ganglioglioma and pleomorphic xanthoastrocytoma. PA might contain foci of oligodendroglioma-like or diffuse astrocytoma-like features. Recently described pilomyxoid variant of pilocytic astrocytoma (PMA) demonstrates piloid cells within markedly loose fibrillary and myxoid background and lacks Rosenthal fibres or EGB, characteristic for classical PA. Tumour cells in PMA are generally arranged radially around blood vessels resembling the perivascular pseudorosettes in ependymomas. Some tumours revealed intermediate features of both PMA and PA.

In some cases the histological features of anaplasia including nuclear atypia, necrosis, mitoses and vascular proliferation could be encountered and require careful interpretation. Moreover, some PAs lacking distinct histologic features of anaplasia might behave in aggressive manner and need additional therapeutic treatment.

Various forms of vascular changes could be observed in PAs including vascular hyalinization resembling vascular malformations. In unique cases with advanced angiomatous proliferation mimicking capillary hemangioma a diagnosis of so called angioglioma could be established. The typical glomeruloid changes of vessels with hyperplasia of endothelial cells might suggest diagnosis of high-grade gliomas.

We present the diagnostic dilemmas resulting from unusual clinicopathological features of PAs. The histological pattern of PAs may mimic some other neoplasms including pleomorphic xanthoastrocytoma, diffuse astrocytoma and glioblastoma or even piloid reactive gliosis typicall for long-standing lesions. Distinction from all these lesions bears important therapeutic and prognostic implications.

[A21]

The spectrum of onconeural antibodies in patients with endometrial and breast cancer

Popławska K[1], Szpurek D[2], Paluch A[2], Englert-Golon M[2], Sajdak S[2], Kozubski W[1], Michalak S[3,4]

[1]Department of Neurology, Poznan University of Medical Sciences, [2]Department of Gynecological Surgery, Poznan University of Medical Sciences, [3]Department of Neurochemistry and Neuropathology, University of Medical Sciences, Poznan, [4]Neuroimmunological Unit, Mossakowski Medical Research Centre, Polish Academy of Sciences, Poznan, Poland

Introduction: The evaluation of onconeural antibodies plays a crucial role in definite diagnosis of neurological paraneoplstic syndromes (NPS). Malignancies associated with NPS in female patients include ovarian cancer, breast cancer, lung cancer and myeloproliferative disorders. However, systemic studies on endometrial cancer are missing and only case reports are currently available in literature. The aim of this study was to analyze the spectrum of onconeural antibodies in women with endometrial cancer and to compare it with non-gynecological malignancy represented by breast cancer.

Material and methods: The study included 42 patients with diagnosed endometrial cancer and hospitalized in Department of Gynecological Surgery Poznan University of Medical Sciences in Poznan and 15 patients with breast cancer identified among 1552 subjects with suspicion of NPS and admitted to Department of Neurology at Poznan University of Medical Sciences. Indirect fluorescence (EUROIMMUN, Germany) was performed as a screening test and Western blotting (EUROIMMUN) as a confirmation test for the presence of onconeural antibodies in patients' sera.

Results: The age of endometrial cancer (61.5 ± 9.5 years) and breast cancer patients (54.8 ± 8.5 years) was not different (P = 0.7000). The most frequent neurological deficit in endometrial cancer patients was neuropathy (21.4%), upper motor neuron syndrome was diagnosed in 4.8%, bulbar palsy in 2.4% and coexisting neuropathy with paraneoplastic cerebellar degeneration – in 2.4% of cases. In breast cancer patients we have found paraneoplastic cerebellar degeneration in 33.3%, neuropathy in 13.3%, coexisting paraneoplastic cerebellar degeneration with upper motor neuron syndrome in 6.7% and plexopathy in 6.6% of cases. Anti-neural antibodies were the most frequent among endometrial cancer patients (anti-myelin – 9.5%, anti-MAG – 7.1%) and onconeural antibodies were detected in 7.1% of patients (anti-Tr and coexisting anti-Ma/Ta with anti-CV2). In breast cancer subjects anti-Ri antibodies were identified in 26.6%, coexisting anti-Ri with anti-Yo in 6.7% as onconeural antibodies, and in 20% – anti-neural antibodies.

Conclusions: Endometrial cancer is associated with predominant anti-neural autoimmunity, while breast cancer with well defined onconeural antibodies. Neuropathy is dominant clinical manifestation of NPS in the course endometrial cancer, but paraneoplastic cerebellar degeneration in breast cancer.

[A22]

SMN – a motor neuron insurance for a whole lifespan?

Rafałowska J[1], Sulejczak D[2], Gadamski R[1], Wojda R[1], Chrzanowska H[1], Modrzewska-Lewczuk M[3], Dziewulska D[1,4]

[1]Department of Experimental and Clinical Neuropathology, [2]Department of Experimental Pharmacology, [3]Photography Workshop, Mossakowski Medical Research Centre, PAS, Warsaw, [4]Department of Neurology, Medical University of Warsaw, Poland

Introduction: SMN (survival motor neuron) gene plays an important role in ontogenesis and its dysfunctions lead to immaturity of skeletal muscles and motor neurons in spinal cord. As a result of SMN gene mutations the affected cells died and clinical symp-

toms of spinal muscular atrophies (SMA) develop. SMA manifests over a wide range of severity affecting infants through young adults. Most severe SMA form is Werdnig-Hoffman disease which symptoms are present from the period of birth (group I). Physiologically, SMN is a part of multiprotein complex that is found in the cell cytoplasm and nucleus. Except SMN, the complex also contains other 7 proteins named gemins 2-8, and plays an essential role in the formation of small nuclear ribonucleoproteins of particular importance to the motor neuron development. Since SMN gene is necessary for normal motor neuron maturity, a question arises whether its activity is preserved or finishes with the end of ontogenesis. In our investigation we examined expression of SMN and selected gemins in rat spinal cords expecting that its results would make possible not only to answer for this question but also would enable to assess how long after the end of ontogenesis the SMN complex is expressed.

Material and methods: The material consisted of spinal cords from 27 Wistar rats at the age of 1-350 days (9 groups composed of 3 rats at age of 1, 10, 20, 30, 60, 150, 200, 250 and 350 days respectively). Expression of SMN and gemin 2, 3 and 4 were assessed at light microscopy with using of immunofluorescence and immunohistochemical methods.

Results: Expression of SMN in rats at the age of 1 day was very weak. In 10 and 20 days old rats it was more pronounced and increased with the animal age. In rats at the age of 30-350 days SMN immunoreactivity was similar in the all examined groups. The same phenomenon was observed in assessment of gemin expression. The immune label for SMN and gemins was observed in neurons both in anterior and posterior horns of the spinal cord.

Conclusions:

1. In Wistar rats expression of SMN and gemins 2, 3 and 4 is present through the whole animal lifespan.
2. In spinal cord expression of SMN and gemins 2, 3 and 4 is present not only in motor but also in sensory neurons.

[A23]

Morphological and biochemical characterization of brain gliomas

Szczerbowska-Boruchowska M[1], Lankosz M[1], Radwańska E[2], Surówka A[1], Adamek D[2]

[1]AGH University of Science and Technology, Faculty of Physics and Applied Computer Science, [2]Department of Neuropathology, Chair of Pathomorphology, Medical College, Jagiellonian University, Cracow, Poland

Existing networks between biologists, clinicians and physicists are already making impressive progress in X-ray fluorescence (XRF) and infrared (IR) biochemical micro-imaging of cells and tissues leading to the identification of spectroscopic markers of diagnostic relevance. New technologies are thus constantly sought to assist pathologists in this demanding and important clinical area. IR and XRF spectroscopies can deliver a very quick "biochemical fingerprint" of cells and tissues, and it was demonstrated in many studies that these techniques can be used to classify tissues as normal or pathological, as well as for classifying and grading pathological samples. The thin tissue sections of various brain tumour types (mainly gliomas) were studied. The elemental chemical micro imaging was carried out for "homogenous" areas of neoplastic tissues as well as for other characteristic structures like blood vessels or areas of calcification. The XRF maps of elemental distributions were obtained. Synergy of the XRF microspectroscopy and multiple discriminate analysis (MDA) was applied for deconstruction of the samples histopatological structures (neoplastic cells, blood vessels, calcification) based on their elemental content. The MDA was also used to differentiate neoplastic samples according to their histopathological classifications. The method allowed finding the elements of the highest importance for the general discrimination of tumour type. It seems justifiable to suppose that the abnormal reactions related with these elements are a source of the unique elemental signature of different types of brain tumour.

The main biological molecules such as proteins, lipids, nucleic acids and carbohydrates were determined in brain glioma sections using infrared spectroscopy. It will enable to find metallo-organic complexes that occur in neoplastic tissues.

Apart from biochemical composition the morphological features of various tumour types were studied.

The morphometric analysis of glioma sections was performed using the national Institute of Health IMAGEJ computer program. The following parameters of the cell nuclei were taken into account: perimeter, diameter, area, circularity, aspect ratio, roundness. No significant differences were found between diffuse astrocytoma, malignant astrocytoma, oligodendroglioma and anaplastic oligodendroglioma in roundness and aspect ratio. Both parameters are also comparable between glioblastoma multiforme and gemistocytic astrocytoma. The anaplastic astrocytoma cell nuclei reveal the highest roundness and the lowest aspect ratio.

The biochemical and morphometric features of neoplastic tissues may be a very useful tool assisting the process of histopathological diagnosis of tumours especially in difficult or disputable cases.

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[A24]

Krabbe disease – diagnostic problems

Szymańska K[1,5], Ługowska A[2], Laure-Kamionowska M[1], Bekiesińska-Figatowska M[3], Raczkowska B[1], Gieruszczak-Białek D[4]

[1]Department of Experimental and Clinical Neuropathology, Mossakowski Medical Research Centre, Polish Academy of Sciences, [2]Institute of Psychiatry and Neurology, Department of Genetics, [3]Department of Diagnostic Imaging, Institute of Mother and Child, [4]Department of Paediatrics, Warsaw Medical University, [5]Department of Child Psychiatry, Warsaw Medical University, Warsaw, Poland

Globoid cell leukodystrophy (another term for Krabbe disease) is a lysosomal storage disease caused by the deficiency of galactosylceramidase (GALC) [EC 3.2.1.46] also known as galactocerebroside β-galactosidase and is inherited autosomally recessively. GALC degrades galactosylceramide (to ceramide and galactose), psychosine (to sphingosine and galactose), monogalactosyldiglyceride, and lactosylceramide. Another lysosomal enzyme, acid β-galactosidase, also hydrolyzes galactosylceramide, but not psychosine. The latter accumulates in the nervous tissue. The accumulation of psychosine is considered to be the pathogenetic mechanism in Krabbe disease leading to the loss of oligodendroglia and myelin, astrocyte activation, and the formation of multinuclear globoid cells. Deficiency of GALC results in demyelination in both the central and peripheral nervous system.

The lysosomal degradation of sphingolipids depends on sphingolipid activator proteins – saposines. So far, one infantile patient with a mutation in the domain of the prosaposin gene (*PSAP*) coding for the saposin A and a clinical picture similar to Krabbe disease was described.

We report here about our patient who was admitted to paediatric clinic at the age of six months. On admission the muscle tone in extremities was significantly increased and deep tendon reflexes were exaggerated, marked hypokinesis was observed. CSF protein concentration was elevated up to 300 mg/dL. Two brain MR imaging performed at an interval of several months revealed the progress of the disease, general brain atrophy with bilateral white matter dysmyelination in centrum semiovale, pyramidal tract and cerebellum. Obtained results of GALC activity measured in blood leukocytes and cultured fibroblasts were low but not unequivocal (the low border of reference values). Other laboratory tests were in the normal range. His health status had been deteriorating and he died at 15 months. The neuropathologic investigation was performed. It showed the damage to white matter in the cerebral hemispheres and cerebellum, the most serious within the pyramidal tract. The histopathologic changes included extensive demyelination, presence of numerous macrophages and globoid cells within the damaged white matter. The neuropathological result confirms the diagnosis of Krabbe disease, although the enzymatic results were not indicative of this diagnosis. In this case the authors consider whether the clinical picture and results of enzymatic investigation is due to mutations in the *GALC* in *PSAP* genes. It requires further molecular analysis (sequencing of *GALC* and *PSAP* genes).

[A25]

Diagnostic and prognostic significance of R132H mutation of isocitrate dehydrogenase 1 (IDH1) in astro- and oligodendrogliomas grade II

Szymas J[1], Majchrzak H[2], Kaspera W[2], Majchrzak K[2]

[1]Department of Clinical Pathology, University of Medical Sciences, Poznan, [2]Department of Neurosurgery in Sosnowiec, Medical University of Silesia, Katowice, Poland

Heterozygous point mutations in the gene encoding isocitrate dehydrogenase 1 (IDH1) codon 132 are frequently and early event in gliomas. Recently the mouse monoclonal antibody specific for the IDH1R132H mutation was developed. We have investigated 61 primary and recurrent astrocytomas and oligodendrogliomas to demonstrate immunohistochemically mutated IDH1R132H cases. Prospective studies were performed in period between July 2005 – December 2010. We assessed 54 patients with 38 astrocytomas and 16 oligodendroliomas grade II WHO classification. IDH1R132H mutation was found in 14/16 (87%) oligodendrogliomas and in 29/38 (76%) astrocytomas. In their concurrent recurrences 5 anaplastic astrocytomas and 1 gemistocytic astrocytoma were positive for IDH1R132H mutation immunohistochemically. We have found 2 oligodendrogliomas, 9 astrocytomas and 1 recurrent glioblastoma without mutation. All recurrent tumours were marked on the same manner with mutation as primary tumour, independent of their biological malignancy. Presence of IDH1R132H mutation has correlated with significantly better prognosis considering progression free survival (PFS), malignant free survival (MFS) and overall survival (OVS). We have observed malignant progression only in 26% (12/46) of mutated cases and in 60% (6/10) of non mutated cases. Constant immunohistochemical positivity in cases carrying IDH1R132H mutation permits microscopically selective identifications of tumour cells even as single cells. This accuracy offer visualization and further morphological sub-classification of tumours according to their secondary structures. This method offers also a new tool for study of IDH1R132H mutated glioma cells in brain environment.

[A26]

A case of ganglioglioma with progression to giant cell glioblastoma

Taraszewska A[1,5], Andrychowski J[2,4], Szopiński R[3], Czernicki Z[2,4]

[1]Department of Experimental and Clinical Neuropathology, [2]Department of Neurosurgery and [3]Laboratory of Photographic Documentation, Mossakowski Medical Research Centre, Polish Academy of Sciences, [4]Department of Neurosurgery, II Medical University of Warsaw, [5]Department of Pathomorphology, Bielanski Hospital, Warsaw, Poland

Gangliogliomas are generally considered to be benign and slow-growing tumours, exhibiting histolo-gical characteristic of WHO grade I or II features. Gangliogliomas of higher grade malignancy occur infrequently as *de novo* tumours or the recurrent tumours with secondary malignant transformation of previously low grade gangliogliomas. Progression of ganglioglioma into glioblastoma is a particularly rare finding.

Here, we report the case of a 26-year-old woman who presented with a tumour of the right temporal lobe. The tumour was surgically removed and histopathologically diagnosed as atypical ganglioglioma (WHO G II). The patient underwent postoperative radiotherapy and remained in a good health during the 4.5 years of follow-up. After this time the control MRI revealed a small local tumour recurrence. However, the next MRI done 5 months later showed marked progression of the tumour and their extension into the optic radiation. The second operation with the right temporal lobectomy and subtotal tumour resection was performed after 5 years from the surgery of primary tumour. Histopathological examination of recurrent lesion revealed highly pleomorphic tumour exhibiting frequent multinucleated and giant cells with bizarre nuclei, mitotic figures, prominent microvascular proliferation and high proliferative activity of tumour cells, assessed by Ki-67 labelling index (about 50%). Tumour cells were immunopositive for glial fibrillary acidic protein, negative for synaptophysin and expressed p53 nuclear immunoreactivity, predominantly in giant cells. Histopathological diagnosis of the recurrent tumour was giant cell glioblastoma (WHO G IV).

[A27]

Cerebral ventricular empyema caused by *Streptococcus intermedius*. Case report

Taraszewska A[1,4], Warzecha A[3], Powała A[4], Baraniecka J[1], Głowacki M[2,3]

[1]Department of Experimental and Clinical Neuropathology, [2]Department of Neurosurgery, Mossakowski Medical Research Centre, Polish Academy of Sciences, [3]Department of Neurosurgery, [4]Department of Pathomorphology, Bielanski Hospital, Warsaw, Poland

Cerebral ventricular empyema (CVE), known also as pyocephalus or pyogenic ventriculitis, is a rare but frequently fatal form of intracranial infection. It can arise by extension of purulent cerebritis or meningitis, rupture of brain abscess, by hematogenous spread, from a contiguous focus of infection or as a result of direct inoculation during head trauma or neurosurgical procedures. Clinical course of CVE may be nonspecific and often indolent resulting in delayed diagnosis and fatal outcome of the disease.

We report a case of previously healthy 38-year-old man, who complained severe headache for 3 days and presented with vomiting and nuchal rigidity. He was alert and well oriented at admission. CT and MRI of the brain revealed focal lesion in the right occipital lobe, penetrating to the lateral ventricle. Lumbar puncture showed purulent CSF and *Streptococcus intermedius* was identified in CSF cultures. The patient worsened suddenly in the 6-th day of hospitalization. He dead 8 days after presentation and 2 days after surgically performed evacuation of the right occipital cerebral abscess. General autopsy revealed purulent endocarditis and myocarditis and septic emboli in pulmonary vessels. Neuropathological examination of the brain demonstrated extensive purulent ventriculitis with massive pus accumulation in the lateral, III and IV ventricles, accompanied by purulent meningitis predominantly about the brain stem.

This case represents a rare report of the fatal infection caused by *Streptococcus intermedius*, manifested as CVE associated with infective endocarditis.

[A28]

Differential expression of calcium-binding proteins in the cerebellum of pups of ethanol-treated female rats

Wierzba-Bobrowicz T, Lewandowska E, Stępień T, Szpak GM

Department of Neuropathology, Institute of Psychiatry and Neurology, Warsaw, Poland

Calcium is an important moderator involved in the regulation of numerous neural functions. The presence of three calcium-binding proteins, calbindin D28k, calretinin and parvalbuminin, has been reported in the cerebellum of pups of ethanol-treated dams (during pregnancy and/or lactation).

In the cerebellar cortex from the pups of control groups all Purkinje cells with their processes were positive for calbindin D28k, but only occasionally for calretinin and parvalbumin. In the experimental groups the calbindin D28k immunoreactivity was detected only in some Purkinje cells, while calretinin and parvalbumin were visible in single Purkinje cells. In the molecular layer in the cerebellum of control groups, stellate and basket cells were positive for parvalbumin, but in experimental groups these antibodies were detected only in some basket cells. In none of the control or experimental group calbindin D28k and calretinin were detected in these cells. In the granular layer in the cerebellum, Golgi, Lugaro and unipolar brush cells were positive for calretinin. Golgi cells were positive for all the examined calcium-binding proteins. The number of positive cells and intensity of immunoreaction for calbindin D28k and parvalbumin decreased in all experimental groups.

The most significant decrease in immunoreactions was observed in the experimental groups of females treated with ethanol during pregnancy and lactation. The immunoreaction for calretinin was detected only in interneurons and it was more intense in experimental than in control groups, particularly in unipolar brush cells.

This observation suggests a possible correlation between the duration of ethanol intoxication and expression of calcium-binding proteins.

[A29]

Expression analysis of precursor cell markers in childhood medulloblastoma

Zakrzewska M[1], Grešner SM[1], Zakrzewski K[2], Polis L[2], Zalewska-Szewczyk B[3], Liberski PP[1,2]

[1]Department of Molecular Pathology and Neuropathology, Chair of Oncology, Medical University of Lodz, [2]Department of Neurosurgery, Polish Mother Memorial Hospital Research Institute, [3]Department of Pediatric Oncology, Hematology and Diabetology, 1st Chair of Pediatrics, Medical University of Lodz, Lodz, Poland

Medulloblastoma (MB) is the most frequent type of embryonal tumours in paediatric population. The pathogenesis of this entity is still unclear and two sources of potential tumour precursor cells, the external granule layer (EGL) and cerebellar ventricular zone (CVZ), are considered. The presence of precursor cell molecular markers for each region was suggested, but their expression profile and potential links with prognosis have not been analyzed yet. In this work we analyze expression of plausible biomarkers of progenitor cells in medulloblastoma, *SOX2*, *PROM1*, *FUT4*, *ATOH1*, *OTX1*, *OTX2* and *NGFR*, their associations with demographic and clinical data, and possible role in predicting outcome. Forty eight children (27 males and 21 females, aged from 5 months to 17 years) were included in this study. The expression level of genes was measured by quantitative real-time PCR with commercially available cerebellar RNA. Univariate Cox proportional hazards models were used to determine the contribution of the gene expression. The genes, that were significantly related with survival ($p \leq 0.02$), were included in a multivariate Cox proportional hazards regression to calculate risk score (RS) for each patient. The univariate analysis of gene expression identified three genes with significant prognostic value, *PROM1*, *OTX1* and *ATOH*. On the basis of the expression levels of these three genes, an original formula for the prediction of outcome was generated.

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[A30]

MGMT mRNA expression in glioblastoma

Zawlik I[1], Piaskowski S[1], Zakrzewska M[1], Jesionek-Kupnicka D[2], Szybka M[1], Kordek R[2], Liberski PP[1]

[1]Department of Molecular Pathology and Neuropathology, Chair of Oncology, Medical University of Lodz, [2]Department of Oncological Pathology, Chair of Oncology, Medical University of Lodz, Lodz, Poland

O^6-methylguanine DNA methyltransferase (MGMT) is a key DNA repair enzyme that specifically removes promutagenic methyl groups from the O^6 position of guanine. It has been shown that *MGMT* methylation and low MGMT expression was associated with better clinical response of glioblastoma to chemotherapy and improves patient's survival irrespective of treatment.

In this study 32 patients with primary glioblastoma were examined for *MGMT* mRNA expression by using real-time PCR. Reduced *MGMT* gene expression was detected in 70% of glioblastoma patients. There was no correlation between *MGMT* mRNA expression and patient's survival. Patients with reduced *MGMT* mRNA level were significantly older (56.6 years old) than patients with normal level (47.1 years old; $P = 0.0441$). Our data indicate that *MGMT* mRNA expression does not affect patients' prognosis, however, may has impact on glioblastoma onset.

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[A31]

Quantitive analysis of astroglia in frontal lobe of fetuses with Down syndrome

Zdaniuk G, Wierzba-Bobrowicz T, Szpak GM, Stępień T

Department of Neuropathology, Institute of Psychiatry and Neurology, Warsaw, Poland

Down syndrome (DS) is the most common chromosomal disorder caused by autosomal aneuploidy of human chromosome 21. DS individuals are affected by various abnormalities but mental retardation is the most significant anomaly. The defective neurogenesis and reduced number of neurons in the development of central nervous system (CNS) are believed to be the major cause of mental retardation. Astro-

cytes, the major cell population in the brain, play a pivotal role in CNS development, but the knowledge of mechanisms underlying the generation of astroglia is still fragmentary. Recent studies have revealed that astrocytes perform a number of functions. The role of astrocytes in the CNS development still needs to be determined, however, we already know that they promote the myelinating activity of oligodendrocytes. They are also involved in the regulation of CNS synaptogenesis by releasing factors, which influence the synapse development. The aim of this study was to investigate the relationships of radial glial cells and astrocytes in the CNS of DS foetuses and compare them with age-matched control. We examined 24 brains derived from human foetuses between 18 and 20 gestation weeks (GW), including 12 foetuses with genetically confirmed Down syndrome and 12 foetuses without obvious developmental or neuropathological abnormalities. The haematoxylin-eosin (H-E) method was used for routine staining. For immunohistochemical staining glial fibrillary acid protein (GFAP) and vimentin antibodies were used. The quantitive analysis of astroglial cells was carried out in the frontal lobe, opposite to ganglionic eminence, along lateral ventricle. The number of GFAP positive cells in DS brains was compared with that in the age-matched controls. We observed substantially increased number of GFAP positive cells in all age range samples of DS brains as compared with controls. The number of radial glial cells was also assessed. We observed a higher number of radial glial cells in DS brains than in age-matched controls with exception to 20 GW, which may indicate earlier ma-turation of DS individuals. Astrocytes in DS brains seemed to be more mature than in controls of corresponding age. No difference in vimentin staining between DS brains and controls was found. It is a well known fact that the dysfunction of astrocytes plays an essential role in the pathogenesis of CNS disorders. Most recent studies have pointed out that the imbalance between neuron cells (decreased number) and astroglia cells (increased number) can lead to cognitive impairments. Taking into consideration the role of astroglia during development we believe that any change in its quantity, reduced or increased, can affect CNS development and lead to disturbances of both neurogenesis and synaptogenesis. Altered astrogliogenesis may be one of the major causes of mental retardation in DS. This is a relatively new approach and only few papers, which address this problem, have been published to date.