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[A1]

Dementia as an element of neurodegenerative conditions other than Alzheimer's disease, in the example of progressive supranuclear palsy and spinocerebellar ataxia

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Neuropathological examinations of cases in which dementia is only a part of a broader neurological syndrome are more "interesting" but on the other hand it forces us to make much greater diagnostic effort and makes the interpretation of both the clinical and neuropathological picture much more complicated.

The authors present a case of progressive nuclear palsy (PSP) (woman, 80) and a case of spinocerebellar ataxia (SCA) (male, 67). In both of them symptoms of dementia coincided with "more specific" manifestations of disease.

In the case of PSP neuropathological examination showed the typical picture of changes for this condition (among others tau-positive tangles in neurons of nucleus locus coeruleus, nuclei of cranial nerves III and IV, superior collicles of lamina quadrigemina, and in periaqueductal grey matter) though not revealing other pathological features which could explain symptoms of dementia (especially Alzheimer-type changes in hippocampus, and entorhinal cortex, or in other regions of the neocortex). In contrast, in the case of SCA apart from characteristic changes in the cerebellum, medulla, and spinal cord, numerous senile plaques, neurofibrillary tangles, and neuropile threads were found in the hippocampus and entorhinal cortex.

In the case of PSP the differential diagnosis must include among others Alzheimer's disease and other neurodegenerations. In the case of SCA with concomitant symptoms of dementia and neuropathological Alzheimer type changes the interpretation should include either co-occurrence of SCA plus Alzheimer's disease or unusual (novel?) type of SCA (the molecular genetic studies are not entirely finished but so far SCA types 1,2,3 and 6 have been excluded).

[A2]

Severe cerebral amyloid angiopathy in dementia with Lewy bodies. Immunohistochemical and ultrastructural study

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Cerebral amyloid angiopathy (CAA) is a clinico-pathological condition caused by the deposition of amyloid in the walls of leptomeningeal and parenchymal vessels. CAA has a fundamental aspect of pathology of many disorders causing dementia. Using immunohistochemical (IHC) staining methods with antibodies to amyloid β protein, α-actin, collagen III, collagen IV and CD34 as well as ultrastructural methods meningeal and cortical cerebral vessels were examined in order to determine vessel wall changes in severe CAA. For that reason we investigated paraffin-embedded sections of intracerebral and meningeal vessels in frontal and temporal region from one case of common type dementia with Lewy bodies (DLB) and two age-matched control cases without the existence of CAA. In the control cases α-actin was deposited only in the media of meningeal and cortical vessels. Collagen IV was present in the media of meningeal and cortical vessels, similarly in capillaries. Collagen III was observed in the adventitia of meningeal vessels and only in superficial cortical vessels.

It was totally absent in deeper cortical arterioles and in capillaries. In a DLB case with concomitant severe CAA, amyloid β overloading was consistently observed in the muscular and adventitial layer of the meningeal and cortical vessels with fragmentation of the media and degeneration of smooth muscle cells. In some vessels increased amounts of collagen III and IV in the thickened media of meningeal and cortical vessels were observed. In others decreased amounts of collagen IV, but not collagen III, in the media were seen, frequently with concomitant thickening of intima and prominent narrowing of the vessel lumen. The broadened intima showed immunoreactive material indicating a marked deposition of collagen III and especially collagen IV. In many vessels nearly total absence of collagen III and IV in their wall was visible, with weak immunoreactivity in the intima, just near the lumen. Capillaries showed increased segmental immunostaining of collagen IV and collagen III. Many meningeal and cortical vessels showed complete loss of α-actin immunoreactivity of the media and positive immunoreactivity of the intima, just near the vessel lumen. "Vessel-within-vessel" changes indicated very severe angiomyopathy with replacement of intima by a thin muscular layer, surrounded by an empty broadened space instead of fibrosed intima, very thin atrophic media with absence of α-actin fibres and atrophic adventitia. The endothelial cell layer did not show any changes with regard to expression of CD34 but degenerative changes of endothelium were seen in the ultrastructural study. In conclusion, our results show that in the response to amyloid loading the vessel media degenerates with concomitant atrophy of collagen IV fibres and deposition of collagen type III with resultant weakening of the vessel wall. The broad vascular intima appears fibrotic with deposition of collagen III and especially collagen IV. In very severe CAA collagen III and IV is visible only in the inner part of the intima just near the vessel lumen and "vessel-within-vessel" changes are observed. The translocation of the media to the intima seems to be a trial of a reconstruction of the functionally most important component of the vessel structure.

[A3]

The role of trace elements in the pathogenesis and progress of pilocarpine-induced epileptic seizures

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The causes of epilepsy, being one of the most frequent neurological diseases, in most cases are still unknown. Trace metals, such as Ca, Mn, Fe or Cu, may play an important role in the processes leading to the atrophy and death of nerve cells in neurodegenerative disorders, among others Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis. Because epileptic seizures induce neurodegenerative changes in selected areas of the brain, it is suspected that trace metals may have a role in the pathogenesis and progress of epilepsy as well.

A topographic and quantitative elemental analysis was carried out for selected areas of rat brain. In the investigations the pilocarpine model of epileptic seizures was used. The metal content was examined using X-ray fluorescence microscopy.

Statistically significant differences in the accumulation of some elements were found between animals with pharmacologically induced epilepsy and naive control rats. The level of calcium was higher in CA1 and CA3 areas of the hippocampus as well as in the brain cortex in the case of epileptic animals. The opposite relation was observed for copper in DG and for zinc in CA3 and DG areas of hippocampal formation.

[A4]

Response of the middle cerebral artery to selected vasoconstrictors after experimental subarachnoid haemorrhage

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Purpose: The main consequence of subarachnoid haemorrhage, for those who survive bleeding, is delayed, persistent constriction of extraparenchymal blood vessels which occurs between the third and seventh day after the insult and results in symptomatic brain ischaemia in about 40% of cases. This vasospasm is considered to be a major cause of patients' disability afterwards. Despite extensive experimental and clinical research, mechanisms of vasospasm are not fully understood. This study aimed to assess dynamics of functional and morphological changes of the middle cerebral artery (MCA) of the rat subjected to subarachnoid haemorrhage (SAH).

Methods: The experiments were performed in adult, male Sprague-Dawley rats according to the protocol approved by the Local Ethical Committee. SAH was induced by puncture of the bifurcation of the intracranial portion of the internal carotid artery. MCA was harvested 24, 48 and 72 hours after SAH and placed in the myograph chamber to study dose-dependent responses to: endothelin-1 (ET-1, 10^{-11}-10^{-8} M), serotonin (5-HT, 10^{-9}-10^{-6} M) and the stable analogue of thromboxane A_2 (U-46619, 10^{-10}-10^{-7} M). At the same time points morphology of the MCA was studied with the light microscope in the sections stained with orcein and van Gieson. The degree of vasospasm was assessed based on the corrugation of the internal elastic lamina (IEL) visualized with orcein . It was expressed as the ratio of the total length of IEL to its internal circumference. Moreover, on consecutive days after SAH, expression of protein Rho A, one of the most important factors which sensitize vascular smooth muscle cells to calcium, was studied with rtPCR.

Results: The most striking differences between control (isolated from rats with sham SAH) and post-SAH MCA were observed 72 hours after SAH. When post-SAH MCA was pressurized to 80 mmHg to develop spontaneous tone it showed oscillations which are characteristic for vasomotion. Such behaviour was never observed in control MCA and was seen rarely (only in 2% cases) in post-SAH MCA at 24 or 48 hours after the bleeding. The reactivity of MCA to ET-1, 5-HT and U-46619 did not differ between control and post-SAH MCA at 24 and 48 hours. It has to be stressed that MCA dilated in response to the lowest dose of each of these vasoconstrictors by about 10%, whereas the highest dose resulted in constriction of MCA by about 35-40% of baseline diameter. The dilatory phase in the case of ET-1 and U-66619 was NO-dependent. At 72 hours after SAH this dilatory phase was absent in post-SAH MCA. The constriction to the highest dose of all three substances was the same as in control MCA. Index of corrugation of IEL increased with time after SAH but the decrease in diameter of the MCA at 72 hours did not increase by more than 30% of control. The expression of mRNA for Rho-A did not differ between control and post-SAH MCA at any time studied.

Conclusion: Our results suggest that at 72 hours after the bleeding in the perforation model of SAH, the middle cerebral artery is in a state of vasomotion which, according to the literature, is the transition phase to permanent increase in tension. This vasomotion is not associated with the increase in the sensitivity of smooth muscle cells to calcium but may be related to the withdrawal of NO-dependent inhibition of the tone. The hypothesis that the inhibition of vasomotion may prevent vasospastic changes deserves further studies.

[A5]

Homocysteine and asymmetric dimethylarginine (ADMA) in the plasma of patients with Alzheimer's disease

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Alzheimer's disease (AD) is accompanied by elevated levels of homocysteine (Hcy). Hcy may induce elevated concentration of asymmetric dimethylarginine (ADMA). Both Hcy and ADMA are amino acids thought

to represent risk factors of vascular diseases. The aim of the study was to estimate plasma levels of Hcy and ADMA together with Met and Arg in AD patients.

The studies were conducted on 26 patients with AD, including 16 women and 10 men aged 34-85 years (72.2±11.2 years). The control group included 35 individuals, 20 women and 15 men, 22-76 years of age (45.1±16.0). Studies were conducted on plasma levels of Hcy and methionine (Met), estimated by HPLC with electrochemical detection, and on levels of ADMA with arginine (Arg), estimated by HPLC with fluorescent detection, in patients with AD and in a control group.

The study showed that after 60 years of age there was a significant increase in plasma level of Hcy ($p<0.01$ as compared to subjects between 22 and 60 years of age) accompanied by an insignificant increase in plasma ADMA. In parallel, the alterations were accompanied in this period of life by lowered levels of Hcy and ADMA precursors, Met ($p<0.05$ as compared to subjects between 22 and 60 years of age) and Arg, in parallel accompanied by a decreased Met/Hcy ($p<0.01$ as compared to subjects between 22 and 60 years of age) and Arg/ADMA levels ($p<0.05$ as compared to subjects between 22 and 60 years of age).

Also, in the patients with AD there were elevated levels of Hcy ($p<0.001$ as compared to the controls) and ADMA, and decreased levels of Met ($p<0.5$ as compared to the controls) and Arg accompanied by decreased Met/Hcy ($p<0.001$ as compared to the controls), and Arg/ADMA ($p<0.05$ as compared to the controls) levels. It was also noted that in AD patients with deep dementia reflected on the MMSE scale only an increased concentration of Hcy was observed (Hcy, $p<0.05$ as compared to the patients with moderate dementia). The remaining analyzed amino acids manifested in general a decreasing tendency as compared to the patients with benign or moderate dementia (except of Arg/ADMA ratio).

The present results indicate that similarly to Hcy, ADMA seems to be a potential risk factor of AD. The results indicate also that developing neurodegenerative disease is accompanied by disturbed metabolism of Hcy and ADMA, and administration of L-arginine, in line with vitamins B6, B12 and folates, to AD patients may offer a modern therapy in neurodegenerative disease.

[A6]

Neuroprotective effects of memantine in selected experimental models *in vitro* and *in vivo*

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Due to unique properties of glutamatergic NMDA receptors, which are ligand-gated Ca^{2+} channels of voltage-dependent sensitivity to inhibition by Mg^{2+}, they play a key role in neuronal plasticity, learning and memory. Abnormal functioning of these receptors may result not only from altered glutamate release, but also from changes in neuronal energy state and excitability. It may lead to cognitive impairment, seen e.g. in Alzheimer's disease (AD). The excessive and/or prolonged activation of NMDA receptors may induce excitotoxic damage to neurons. It is widely accepted that this Ca^{2+}-mediated mechanism plays a key role not only in acute CNS disorders like stroke, epilepsy or spinal cord trauma, but also in neurodegenerative diseases.

Several clinical studies have demonstrated that memantine, which is a low-affinity voltage-dependent uncompetitive antagonist of NMDA receptors and replaces Mg^{2+} in blocking Ca^{2+} influx to neurons, improves cognition, behaviour and everyday activity in moderate to severe AD. Because of the low affinity and rapid off-rate kinetics of memantine at the NMDA channel the physiological glutamatergic neurotransmission is preserved, and therefore memantine is free from the well known psychomimetic properties of high affinity uncompetitive NMDA receptor antagonists and is well tolerated by the patients.

In connection with this the question arises whether memantine effectively blocks excessive pathological activation of NMDA receptors and prevents excitotoxic neuronal damage in vitro and in vivo. To respond to this question, the examples of neuroprotective activity of memantine in *in vitro* and animal models including own data will be presented and discussed. Our results demonstrated that memantine, especially in combination with antagonists of group I mGluRs, effectively inhibits homocysteine-induced neurotoxicity in cultured cerebellar granule cells, and applied systemically to Mongolian gerbils in doses corresponding to those

therapeutic in AD prevents loss of CA1 pyramidal neurons evoked by transient global ischaemia, without interfering with the mechanisms of tolerance induced by preconditioning. Data from the literature suggest that memantine may also induce neuroprotection in Alzheimer's patients.

[A7]

Atypical morphological changes in the thalamus in a patient with frontotemporal dementia with motor neuron disease and parkinsonism

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We report a case of frontotemporal dementia with motor neuron disease and parkinsonism (FTD-MND-P) in a 65-year-old man with a 5-year clinical course of the disease. The diagnosis was made based on clinical symptoms and the finding of ubiquitin-positive, tau and alpha-synuclein negative intraneuronal inclusions in the frontotemporal cortex, dentate gyrus and anterior horns of the spinal cord. Unusual features included spongiosis, severe neuronal loss with microgliosis and granulovacuolar degeneration (GVD) in the thalamus and, to a lesser extent, globus pallidus. In these structures, very intensive accumulation of lipofuscin-like material in neurons and neurolysis were also found. Immunohistochemical reactions revealed lack of pro-apoptotic Bax and caspase-3 expression. A part of neurons with GVD revealed immunoreactivity to beclin-1 and cathepsin D. Since beclin-1 and cathepsin D are regarded as markers of autophagy, we conclude that the loss of neurons observed by us in the affected brain regions might be connected with disturbances in autophagy. We also consider that although the pathophysiological relationship between FTD-MND-P and the involvement of the thalamus and globus pallidus was unclear, it might be more than coincidental and suggests a potential disease continuum among frontotemporal lobar degenerations.

[A8]

Ultrastructure of cardiomyocytes in some genetically determined cardiomyopathies

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An ultrastructural study of cardiomyocytes in genetically determined cardiomyopathies is presented.

The disarrangement of the sarcomeric pattern in myocytes of a child with congenital cardiomyopathy was the hallmark of titin deficiency. Overexpression of defective protein in myocytes was a specific marker of mutation in the desmin gene. The accumulation of early and late autophagic vacuoles in myocytes of a 19-year-old boy was a cause of LAMP-2 deficiency and mutation in a gene mapped to the chromosome Xq24. The most surprising finding observed in dilated cardiomyopathy with lamin AC deficiency was the structural remodelling of myocyte nuclei. All these data indicate that ultrastructural analysis of cardiac biopsies facilitate the right diagnosis.

[A9]

Experimental allergic encephalomyelitis (EAE) in rats

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Experimental allergic encephalomyelitis was induced in 22 female rats (Wistar) 180-200g of weight immunised with homogenate of the spinal cord of guinea pig, which was diluted with phosphate buffer (in proportion 1:1). It was mixed with a complete Freund's adjuvant, and 4 mg/ml of a culture of mycobacterium tuberculosis was added. A ready homogenate was administered to the bulbous of both hind legs in the amount of 100 µl/leg.

Animals used in the experiment were divided into three groups. The rats belonging to the first group (8) were only immunised. The rats from the second (6) and third (8) groups 10 days before immunization received hydrolysate orally, done from lyophilized spinal cord of domestic pig diluted in the proportion of 20 mg of hydrolysate to 0.5 ml of buffer. Every two days the rats were given four doses in total. Each dose consisted of 20 mg/kg in the second group and 100 mg/kg in the third group. Within 14 days after immunization transcardial perfusion with 4% solution of formalin was carried out under deep anaesthesia with chloral hydrate, then the spinal cord was taken out. After dehydrating and embedding in paraffin the cords were cut into 8 µm sections. The specimens taken from the cervical, thoracic and lumbar parts of spinal cord were stained with cresyl violet and with immunocytochemical methods using GFAP, vimentin, albumin S100 and LB4.

As a result of the immunization, inflammatory infiltrates appeared in the tissue of spinal cord. They were mainly localized perivascularly, more often in the white matter than in the grey matter. The number of infiltrations showed variability in the groups of animals, as well as in the respective parts of the cords. The intensity of GFAP and vimentin reaction was higher in cases with numerous infiltrations. This correlation was not observed in albumin S100 and LB4. The computer program GIMP version 2.4.5 was used to evaluate the modulator effect of orally given hydrolysate of pig cord on the intensity of inflammatory infiltrations. The results of the morphometric analysis showed the lack of therapeutic effect of orally given hydrolysate on the intensity of infiltrations. A slight influence on the increase of infiltrations was observed with a dose of 100 mg/kg. Further studies on the effect of low dose of spinal cord pig hydrolysate (5 mg/kg) are necessary. The dose range of antigen is important in inducing oral tolerance.

[A10]

Volumetric analysis of brain atrophy on MRI in relation to the IGF-1 level in patients with dementia

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Patients with dementia continue to lose brain tissue at a much more rapid rate than normal patients of the same age. In the standard autopsy examination of the brain the main attention is given to regional neocortical atrophy, atrophy of the hippocampus, entorhinal cortex and ventricular enlargement. Hippocampal atrophy is now considered as the most characteristic and early feature of brain atrophy recognized on MRI in AD. IGF-1, the principal member of the IGF family, is known to protect against cell loss in neurodegenerative disorders. Since inadequate levels of IGF-1 could be responsible for progressive neuronal death and loss of synapses, the indirect but main pathology which leads to the clinical features, it may be a target factor in searching for the cause of brain atrophy.

The first stage of our research was the volumetric analysis of the brain regions using MRI in patients with dementia including AD and VaD. Plasma samples for IGF-1 analysis were stored at -80°C until processing and the levels of IGF-1 were determined using ELISA. The peripheral levels of IGF-1 were investigated and correlated with the brain atrophy.

MRI revealed that among patients with dementia (AD and VaD) as well as in patients with AD only, brain atrophy including hippocampal atrophy increased. Subsequent analysis showed that the IGF-1 level influenced the atrophy of some brain regions in patients with dementia and VaD but not in AD patients. Importantly, higher IGF-1 serum levels were responsible for the less advanced brain atrophy.

Finally we conclude that IGF-1 was shown to protect against brain loss on MRI in patients with dementia.

[A11]

Combination of genetic factors increases risk for AD: *PRNP*, *PRND*, *CYP46* and *APOE* study

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Alzheimer's disease (AD) is a complex neurodegenerative disease influenced by genetic as well as environmental factors. To date over 200 genes have been examined for their potential role in AD; however, the *APOE* gene polymorphism still remains the only undoubted risk factor for sporadic late-onset AD. Numerous other genes have been considered as putative risk factors, but none of them have been definitely confirmed. Here we present an analysis of 8 polymorphisms within *APOE*, *PRNP*, *PRND*, and *CYP46* genes in 213 Polish late-onset AD patients and 171 non-demented elderly controls. The analyses were performed using PCR-RFLP method or DNA sequencing. Carriers of the ε4 allele were overrepresented in AD patients compared to the controls only when this allele was accompanied by other factors. Moreover, the examined risk factors seemed to show synergistic interactions. The highest risk for AD (26-fold) was found for individuals who co-inherited *APOE* ε4 allele, *PRNP* codon 129 homozygosity, *PRND* codon 174 Thr allele, and *CYP46* rs754203 g allele. AD can be influenced by genetic profiles leading to appearance of the disease, composed of genes which separately evoke a small or unnoticeable effect. Testing of genetic patterns instead of single genes/polymorphisms might be a better way to identify risk alleles. The study was supported by Polish State Committee for Scientific Research grant No. PBZ-KBN-124/P05/2004.

[A12]

Diagnostic difficulties in cases with extensive cortical dysplasia in patients with intractable epilepsy

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Cortical dysplasia associated with medically intractable temporal epilepsy exhibits various neuropathological presentation. The most common form of cortical dysplasia is FCD type II, characterized by distinct cortical dyslamination accompanied by neuronal pathology. Two subtypes of FCD, type IIA with dysmorphic, bizarre neurons in grey and/or white matter, and type IIB (Tylor-type) with presence of so-called "balloon cells", have been established.

We presented the cases with extensive neuronal-glial abnormalities detected in biopsy specimens from cortical resections due to pharmacoresistant epilepsy. Massive disorganization of cortical layers with neuronal dyslamination and groups of large dysmorphic neurons, including so-called "balloon cells" typical for FCD type IIB, were seen. Cells with intermediate phenotype between dysplastic neurons and balloon cells were also encountered. Immunohistochemical studies revealed expression of both neuronal and glial markers in some balloon cells. The advanced neuronal abnormalities were accompanied by prominent proliferation of astroglial cells that might resemble true neoplastic processes of well differentiated low-grade astroglial tumour. Within some areas of the cortical lesions both neuronal and glial abnormalities shared the main features of mixed neuronal-glial tumour such as a ganglioglioma.

It could be suggested that the distinction between extensive dysplastic cortical changes and neuronal-glial neoplastic transformation is sometimes unclear.

[A13]

Analysis of polymorphic sites in *APBB2* and *NEP* genes in Alzheimer's disease

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The steady-state level of β-amyloid (Aβ) represents a balance between its biosynthesis and degradation, processes dependent on the activity of several cellular proteins. The disturbance of this equilibrium may result in pathological Aβ processing occurring in Alzheimer's disease (AD). The products of *APBB2* and *NEP* genes are involved in metabolism of amyloid precursor protein (APP). *APBB2* is an adaptor protein possibly participating in Aβ synthesis, while *NEP* takes part in degradation of β-amyloid. Previous investigations demonstrated that overexpression of *APBB2* promotes Aβ formation, whereas decreased *NEP* activity may contribute to the accumulation of β-amyloid and development of AD. In this study we analyzed three single nucleotide polymorphisms (SNPs) within the *APBB2* and *NEP* genes in 213 Polish late-onset AD patients and 170 non-demented elderly controls. The analyses were performed using the PCR-RFLP method. We found no difference in the *APBB2* hCV 1558625 and rs13133980 SNP genotype frequencies between AD and controls; therefore, these polymorphisms do not seem to influence the risk for AD. On the other hand, *NEP* rs701109 CC genotype seems to be associated with AD, but further analysis is needed to confirm these results.

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[A14]

Failing brain glucose metabolism in Alzheimer's disease and its animal model

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Whereas a decrease of brain glucose uptake is undoubtedly an early and characteristic sign of Alzheimer's disease (AD), a controversy remains as to whether it reflects a cause, or is a result of, the disease process. The most popular "amyloid cascade" hypothesis assumes that a primary causative factor in AD is overproduction of amyloid beta, which is toxic toward neurons; when neurons degenerate, brain glucose uptake falls. On the other hand, it has been hypothesized that a primary causative factor in AD is brain insulin receptors' desensitization and a consequential fall in the brain's ability to consume glucose; according to this concept AD may be described as a brain-specific (type 3) diabetes. While the aforementioned hypotheses are not mutually exclusive, each of them is a starting point for the development of very different treatment modalities, e.g. amyloid fibrillization inhibitors or vaccination vs. development of an alternative fuel for brain energy metabolism. Adult mammalian brain can partially switch to ketone bodies as the principal energy source (e.g. during starvation), but it remains to be shown whether brain chemistry and structure can be maintained over a long time on this alternative fuel. A model of rat brain insulin receptors' desensitization, i.e. brain glucodeprivation, has been developed, based on intracerebroventricular injections of diabetogenic toxin streptozotocin (icv STZ). In our current studies this model has been adopted to monitor changes in brain chemistry by nuclear magnetic resonance spectroscopy. Sixteen metabolites were quantified from 27 microL volume in a brain region which included the hippocampus and cerebral cortex, prior to, 3 weeks after and 2 months after icv STZ. Metabolite concentrations in control animals were in agreement with the biochemical literature, whereas following icv STZ increase in lactate and progressive decrease of glutamate, N-acetylaspartate, total creatine (Cr+PCr), glutamate plus glutamine, choline compounds and macromolecules (lipids, proteins) was found. The icv STZ model appears to be well suited to test metabolic efficacy of substituting glucose with ketones as principal metabolic fuel for brain tissues. The other aspect of our

studies concerns the assessment of influence of icv STZ on brain DYRK1a protein. This kinase has recently been implicated as a possible link between amyloid beta overexpression and tau protein hyperphosphorylation, and its pharmacological inhibition has been pointed out as a possible treatment to prevent/retard dementia.

[A15]

HER2 expression in neuroblastic tumours

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Current risk-based therapy in neuroblastoma is effective in patients with low or intermediate risk features; however, treatment of advanced tumours in children over one year of age is not satisfactory. Expression of epidermal growth factor receptor HER2 is associated with aggressive behaviour in some neoplasms in adults. The aim of the study was to examine relations between HER2 expression and patho-clinical characteristics of neuroblastic tumours.

102 cases of neuroblastic tumours were evaluated. The analyzed data included: age, stage, primary tumour localization, histological diagnosis, NMYC status and patients' survival. The series included 64 tumours untreated before sampling and 36 sampled after inductive chemotherapy.

Immunohistochemical labelling with monoclonal antibody anti-c-erbB2 on tumour sections from paraffin blocks was performed. The staining was considered positive if more than 1% of neoplastic cells were immunoreactive. HER2 expression score was assessed based on intensity of reaction and percentage of positive cells, and was graded 1+, 2+ or 3+.

HER2 expression was membranous and/ or cytoplasmic. 20 cases were HER2-negative, 88 HER2-positive: HER 1+ in 32, HER 2+ in 30 and HER 3+ in 20 cases.

Statistical analysis showed a significant correlation between HER 2 expression and tumour histology – strong labelling and prevalent +2 and +3 in differentiating neuroblastoma and ganglioneuroblastomas. The relation of higher HER expression and tumour differentiation with inductive chemotherapy was also found. A borderline relation of HER2 negativity with risk of mortality (p=0.07) and NMYC amplification (p=0.09) was revealed.

Our observations show that HER2 expression in neuroblastic tumours seems to depend mainly on the status of differentiation of the neoplastic cells.

[A16]

Contribution of vascular bed components in development of Schwannian stroma in neuroblastic tumours

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Neuroblastic tumours (NB) are a heterogeneous group of neoplasms including neuroblastoma, ganglioneuroblastoma and ganglioneuroma. Diagnostic criteria in NB are based on the level of neuroblastic cell differentiation and Schwannian stroma amount. The origin of Schwannian stroma is an unresolved question, but it may be related to tumour vascularization.

The study was performed on archival tissue samples from 65 neuroblastoma (NB) Schwannian stroma-poor tumours including 44 untreated and 21 chemotherapy pre-treated tumours. The series was composed of 5 undifferentiated NB, 22 poorly differentiated NB, 22 differentiating NB, and 16 ganglioneuroblastomas.

The morphological analysis included examination of tumour vascular pattern in H-E and immunohistochemical assessment of sections with CD34, ASMA and

S-100 to examine the development and components of Schwannian stroma.

Three types of vascularization were observed – reticular (delicate stroma surrounding small tumour nests – 18 NB), trabecular (stroma separating large tumour sheets – 16) and irregular – 31 (including all GNB). Vascular changes comprised fibrosis, hyalinization and in 22 cases microvascular proliferation. Evident colocalization of beginning/developing Schwannian S-100 positive stroma and vascular framework was found – first S-100+ cells were located exclusively perivascularly. Extraendothelial CD34 expression characterised perivascular and stromal stellate/ bipolar cells in NB stroma-poor cases and endoneurial fibroblasts in GNB. ASMA+ cells were periluminal, especially numerous in MVP and extravascular in developing young Schwannian stroma. In ganglioneuroblastoma they were scanty.

Our analysis showed remodelling of the vascular pattern parallel to increasing NB maturation: reticular – trabecular – irregular. Moreover, we observed perivascular CD34 and ASMA+ positive cells together with S-100-positive cells in developing Schwannian stroma.

[A17]

Neuroblastoma with opsoclonus-myoclonus-ataxia syndrome (OMA) –immunomorphological analysis of tumours

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Opsoclonus-myoclonus-ataxia (OMA) syndrome (Kinsbourne's encephalopathy) is a rare neurological paraneoplastic syndrome. In children OMA usually precedes the detection of neuroblastic tumour. The oncological prognosis is good, because the underlying tumour is most often localized, with differentiating histology and without MYCN amplification. The neurobehavioural prognosis of the patients is however uncertain. Many children have developmental abnormalities – cognitive, language and motor, as well as persistent neurological symptoms.

We report on four cases of neuroblastic tumours with OMA in children treated in the Medical University of Gdansk. The neurological symptoms occurred within 1 m – 6 yrs before the suprarenal gland tumour detection. All patients are alive without signs of cancer and in MRI of the brain no changes are seen. In two children neurobehavioral developmental alterations are present; also viral infections cause recurrence of OMA with need of steroid treatment.

The histopathological examination was performed on paraffin-embedded tumour sections. Immunohistochemical analysis was made based on antibodies against LCA, CD3, CD20, CD56, CD4, CD8, TNFalpha, CD68, HLA-DR, CD21, MHC-1 (Dako, Denmark); En Vision.

Histologically differentiating neuroblastoma, 2 ganglioneuroblastomas and maturing ganglioneuromas were diagnosed. In all cases within the neoplastic tissue there were intense inflammatory infiltrates in the form of diffuse parenchymal, perivascular and/or lymphadenopathic foci. Inflammatory infiltrates were composed mainly of T lymphocytes (CD3+), CD56+ NK cells and smaller populations of B cells, dendritic cells (CD21+) and macrophages (CD68+). The main components of T cell infiltrates were cytotoxic CD8+, which with CD56+ cells invaded and destroyed neuroblastic and ganglliocytic cells. Within germinal centres of lymphoid follicles formation HLA-DR expression was observed. Perivascular infiltrations were made of mixed populations of lymphocytes T and B. TNF alpha labelling was found in the stromal cells – in macrophages and focally in endothelial cells. The neuroblastic and ganglion cells expressed MHC-1 but did not express HLA-DR antigen. Neurological paraneoplastic syndromes are examples of natural antitumour immunity. The tumours linked to these syndromes express onconeural antigens, which activate the immunological reaction focused on the neoplastic tissue and CNS neurons. Performed analyses reveal that the dominant immunological response is of a cellular type in examined neuroblastic tumours.

[A18]

SSPE in an adult presenting with Balint's syndrome and visual deficits coexisting with craniopharyngioma

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Subacute sclerosing panencephalitis (SSPE) is a chronic inflammatory disease of the central nervous system caused by a slow persistent infection with the measles virus. This disease usually affects children with a history of measles, followed by an incubation/latent period of several years. It is a fatal disease with no proven curative treatment available. Nowadays SSPE is a very uncommon disease in developed countries due to anti-measles vaccination. We present an extraordinary case of SSPE in an adult, coexisting with brain tumour.

The clinical presentation of our 31-year old patient was quite atypical with visual symptoms and Balint's syndrome as the main manifestations of disease for about 3 years. The next period of illness, since the occurrence of myoclonic jerks, turned into the fulminant course with progression to stage 4 and death within two and half months.

In neuropsychological examination the patient presented anosognosia, visual and spatial agnosia, ideomotor and ideational apraxia, visual attention deficits and visual hallucinations. All these symptoms created the diagnosis of Balint's syndrome. In the course of the disease he also developed dementia syndrome. Visual symptoms were caused by ocular lesion by chorioretinitis, macular degeneration, optic nerve atrophy and brain destruction as well as a sellar region tumour – craniopharyngioma.

Histopathologically the case was typical for SSPE. The histopathological changes correlate with the evolution of the process recorded in consecutive MR neuroimaging.

The oldest changes with chronic demyelinization and atrophy involved the occipital and parietal lobes. The chronic inflammatory and demyelinating process in the temporal region was florid and in the frontal lobes they were in an active phase of the disease. An interesting finding was the multiplicity of the viral inclusions, confirmed with the ultrastructural examination, related possibly to a very long replication of the viruses.

[A19]

Expression of the 8-α-oxoguanine DNA glycosylase 1 gene (*OGG1*) and level of oxidative DNA damage in peripheral lymphocytes in patients with Alzheimer's disease

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Reactive oxygen species (ROS) are highly reactive and may oxidize macromolecules in cells such as proteins, lipids and especially nucleic acids (DNA). ROS cause several base or sugar modifications in the structure of DNA, leading to strand breaks in the DNA chain.

OGG1 is a main DNA repair enzyme that excises 8-oxo-2'-deoxyguanosine (8-oxo2dG) from DNA. 8-Oxo2dG is one of the crucial lesions produced in DNA by oxygen radical-forming agents. It was postulated that decreased expression of OGG1 may lead to higher background mutation frequency and could increase the risk of DNA damage. Damage of genomic DNA may lead to cell death; in consequence it causes degenerative disorders and mutations resulting in neoplasia and hereditary diseases.

In this study we suggest that different expression of OGG1 in leukocytes from Alzheimer's disease (AD) patients as compared to the control group may be a specific marker of DNA damage in neurodegenerative diseases.

The studies were conducted on 41 patients with AD, including 25 women and 10 men aged 34-84 years. The control groups included 51 individuals: 20 women and 31 men aged 22-83.

Using reverse transcription and real-time quantitative PCR analysis (RQ-PCR), we compared the level of OGG1 (OGG1-1a, -1b and -1c) mRNA isoforms in peripheral blood mononuclear cells (PBMCs) of AD pa-

tients and control groups. The level of 8-oxo2dG was determined using HPLC/EC/UV (high-pressure liquid chromatography system with electrochemical and UV detection) technique and the level of OGG1 protein was determined with the Western Blot method.

We observed an increase of the level of 8-oxo2dG after 60 years of age (insignificant) and in AD patients ($p<0.01$) as compared to the controls and decrease of levels of OGG1-1b isoform ($p<0.01$ in the controls after 60 years of age and $p<0.001$ in AD patients as compared to the controls) with OGG1 protein ($p<0.001$ in the controls after 60 years of age and $p<0.01$ in AD patients as compared to the controls) in the control group after 60 years of age and in patients with AD. The level of OGG1-1c isoform increased only in AD patients ($p<0.001$) as compared to the controls and was the highest in patient with mild dementia (on the MMSE scale). The level of OGG1-1a expression increased only in patients with AD as compared to the controls as well ($p<0.001$).

It is possible that OGG1-1c is involved in pathogenesis of AD and OGG1-1b is important for biosynthesis of OGG1 protein in physiological and pathological conditions.

[A20]

Influence of memantine on levels of oxidative DNA damage and expression of 8-oxoguanine DNA glycosylase (OGG1) in the aging rat brain

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In the aging brain free radicals are generated. Reactive oxygen species are highly reactive and may oxidize macromolecules in cells such as proteins, lipids and especially nucleic acids (DNA) and cause several base or sugar modifications in the structure of DNA, leading to strand breaks in the DNA chain. OGG1 is a main DNA repair enzyme that excises 8-oxo-2'-deoxyguanosine (8-oxo2dG) from DNA. 8-Oxo2dG is one of the crucial lesions produced in DNA by oxygen radical-forming agents. It was postulated that decreased expression of OGG1 may lead to higher background mutation frequency and could increase the risk of DNA damage. Damage of genomic DNA may lead to cell death; in consequence it causes degenerative disorders such as Alzheimer's disease (AD).

Memantine is a new drug in the therapy of AD. Preclinical studies indicated that memantine improves cognitive functions and protects neurons before degeneration. Memantine administrated in neuroprotective doses in experimental animals improved behavioural function.

The aim of the study was to analyse the influence of memantine on the levels of 8-oxo2dG and OGG1 protein.

The studies were performed on 3.0-3.5 – and about 20-month-old female rats of Wistar strain. Memantine was administered in 20 mg/kg/day doses in 20-month-old rats for 14 days. Brains of animals were isolated and divided into 3 structures: cerebral grey matter (GM), subcortical white matter (WM) and cerebellum (C). The level of 8-oxo2dG in analyzed structures of rat brains was determined using HPLC/EC/UV (high-pressure liquid chromatography system with electrochemical and UV detection) technique and the level of OGG1 protein was determined with the Western Blot method.

Our study showed that the level of 8-oxo2dG increases in all analyzed structures of experimental animals with age and decreases to the levels in 3.0-3.5-month-old rats in GM and WM ($p<0.05$) after administration of memantine in 20-month-old rats.

Moreover, the level of OGG1 decreases in all analyzed structures of experimental animals with age and increases in GM after memantine administration in 20-month-old rats.

Memantine in aging rat brain alters oxidative stress (8-oxo2dG) and the repair system.

[A21]

The treatment of intracranial tumours with psychiatric disturbances and dementia syndrome

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Introduction: Symptomatology of cerebral tumours depends on their localisation. Tumours of the frontal lobe can cause mental disturbance, amnesias and dementia syndrome. Often patients are treated for a long time, without proper diagnostic procedures including CT and MRI examination.

Material: During the years 2005-2007 in our department 45 patients with a tumour of the anterior fosse or anterior fosse and paranasal sinuses were treated. There were 24 women and 21 men aged 37 to 80 years. After CT and MRI examination the following localisation of the tumour was reported: 16 tumours – bifrontal; 15 tumours in the frontal, 10 paranasal sinuses with anterior fosse penetration; and 4 tumours – sphenoid wing.

Methods: In all cases surgery was performed. Bifrontal craniotomy was performed in 15 cases, frontal approach in 15 cases, fronto-facial attempt in 10 cases, pterional approach in 4 cases and huge bifronto-temporal in 1 case. One patient was operated on in two steps (frontal approach from the left and right side). Histopathological examination revealed: meningioma in 35 cases and 10 cases of carcinoma. Assessment of psychiatric state and the dynamics of disorders of cognitive functions was made by interview. The Karnofsky Performance Status Scale was used.

Results: Tumours were totally removed in all cases. Neurological and psychiatric worsening were not noted. Clinical state of patients was assessed before and after the operation according to the Karnofsky scale. In 29 cases all psychiatric symptoms withdrew. In 16 cases we noted improvement of neurological state.

[A22]

Molecular classification of tauopathies based on differential tau protein pathology

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A dysfunction of microtubule-associated Tau protein is responsible for neurodegenerative disorders, the so-called tauopathies. Many aetiological factors may disturb Tau biology at different levels of its cellular metabolism: 1/ posttranslational modifications (mainly hyperphosphorylation and/or abnormal phosphorylation of the protein), 2/ *MAPT* gene expression (disturbances of alternative splicing caused by pathogenic mutations and H1 haplotype changing the ratio of 3R tau and 4R tau isoforms), 3/ abnormal protein structure and interactions with tubulin (pathogenic mutations in the *MAPT* gene) and 4/ still unknown environmental factors. Tau protein aggregates differ in both phosphorylation and content of Tau isoforms. The differences can be detected in Western blotting analysis as specific biochemical patterns of tau isoforms. Moreover, there are differences in regional distribution of the set of pathological tau proteins. Distinct sets of isoforms are expressed in different neuronal populations. According to the molecular classification of Tau aggregation, the following five classes of tauopathies can be recognized: Class 0 (frontal lobe degeneration non-Alzheimer non-Pick), Class I (with a major Tau triplet at 60, 64, 69 kDa includes e.g.: AD, ALS/parkinsonism-dementia complex of Guam and FTDP-17), Class II (with a major Tau doublet at 64, 69 kDa and the concept of 4R tau includes e.g.: CBD, PSP and FTDP-17), Class III (with a major Tau doublet at 60, 64 kDa and the concept of 3R tau includes e.g.: Pick's disease and FTDP-17) and Class IV (with a major Tau at 60 kDa includes myotonic dystrophy of type I and II). A better knowledge of both the molecular classification of tauopathies and aetiological factors important for the formation of Tau aggregates is essential for future development of their differential diagnosis and therapeutic strategies.

[A23]

Spinal canal tumours in the material of the Department of Neuropathology, Jagiellonian University Medical College

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Spinal cord tumours constitute a minor part of the CNS neoplasms. Due to their damaging influence on the spine and the spinal roots they are a serious clinical problem and can lead to severe disability.

The aim of this study is to present the material on spinal tumours collected in the Department of Neuropathology in the last ten years.

Two hundred histopathological examinations of spinal tumours were evaluated between August 1997 and August 2007. This group of patients included 104 females and 96 males aged between 18 and 79 years old with a mean age of 57.

Apart from "typical" intraspinal tumours like astrocytoma and ependymoma and the most frequent intracranial extraspinal tumours like schwannoma, meningioma, neurofibroma, in these locations there occur rare neoplastic and non-neoplastic changes. Among those rare conditions are paraganglioma of the filum terminale, myxopapillary ependymoma, different variants of cysts such as enterogenic cyst, dermoid cyst, arachnoid cyst, neoplastic and non-neoplastic bone tumours such as giant cell tumour, chordoma, chondrosarcoma, aneurysmatic bone cyst, and intraspinal metastases.

The precise statistics of the above-listed spinal lesions will be presented and discussed using material of the Department of Neuropathology, Jagiellonian University Medical College.

[A24]

Modern methods for chemical micro-imaging of tissues in neurodegenerative processes with synchrotron radiation

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Altered homeostasis of metal ions seems to play a critical role in neurodegeneration. However, the lack of an analytical technique with sufficient spatial resolution prevents the investigation of metal distribution in neurons. Chemical imaging is the simultaneous measurement of chemical information and spatial information. Special attention is paid to the remarkable opportunities provided by using synchrotron radiation in micro-chemical imaging. The results of application of synchrotron radiation to investigate Parkinson's disease, amyotrophic lateral sclerosis and brain gliomas are presented. Topographic and quantitative analysis with synchrotron radiation based techniques allows the determination of minor and trace elements and oxidation state of selected elements in thin tissue samples of human central nervous system at the single cell level. The comparison between these pathological cases and a control group pointed out differences in elemental composition and oxidation state of Fe and S of the tissues.

[A25]

Neuropathology of neurodegeneration caused by misfolded proteins

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D. Carleton Gajdusek, a Nobel laureate of 1976, stressed that one should always discriminate between the cause and the pathogenesis of the disease. The cause of many neurodegenerative disorders is the ac-

cumulation of abnormally folded (misfolded) forms or isoforms of normal proteins. Those misfolded proteins acquire the β-sheeted conformation and accumulate in the central nervous system (CNS) in the form of amyloid fibrils. It appears that the oligomers of misfolded proteins, smaller than the final fibrils, are neuro(neurono) toxic species.

The final proof that neurodegeneration is caused by misfolded proteins came from multiple discoveries that mutations within genes encoding for precursors of the final deposits (amyloids) actually cause those diseases (Table I). The best examples are prion diseases. In the CNS, and in other tissues depending on the particular disease, there is an accumulation of the abnormal isoform of prion protein, PrP^d, "d" from "disease". PrP^d is mostly β-helical while its normal isoform, PrP^c, "c" from "cellular", is composed of 3 α-helices and 2 short β-strands. However, why and where the conversion takes place is not known. Mutations within the *PRNP* gene that encodes for PrP^c cause familial Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker disease or fatal familial insomnia.

Another more complex situation occurs in Alzheimer's disease, where the final peptide, Aβ or β-peptide, is an internal transmembrane part of a larger β-amyloid precursor protein (βAPP). Two enzymes, β- and γ-secretases, are necessary to cut the sequence of βAPP to release Aβ; γ-secretase is a complex enzyme encoded, in part, by genes presenilin 1 (*PS1*) and presenilin 2 (*PS2*). Mutations in the *PS1*, *PS2* or *βAPP* genes can cause familial Alzheimer's disease (FAD).

The current topic of intense interest in neurodegeneration is fronto-temporal dementias (FTD). A proportion of FTD are caused by mutation within the *MAPT* gene that encodes for MAP-τ which accumulates in the CNS as glial and neuronal tau inclusions (FTLD, fronto-temporal lobar degeneration-tau). Recently yet another subtype of FTLD was discovered in which ubiquitin-positive and MAP-τ-negative inclusions appear. Mutations in the progranulin (*GRN*) gene are responsible for this illness. *GRN* is located on chromosome 17q21-22, in close proximity to the *MAPT* gene. However, within inclusions, another protein accumulates – TAR DNA-binding protein (TARDBP, also known as TDP-43). How mutations within the *GRN* gene lead to accumulations of TDP-43 protein is currently unknown.

The perspectives are unclear. The pathogenesis of neurodegenerations is practically unknown – the only common denominator is autophagy. Despite significant progress in understanding of some basic mechanisms leading to cellular death, more needs to be discovered to fully appreciate the complexities of neurodegenerative processes. Thus, research goes on...

Table I. Neurodegenerations caused by protein misfolding

Disease	Precursor protein	Misfolded protein
Alzheimer's disease	β-amyloid precursor protein (βAPP)	beta peptide (Aβ)
Parkinson's disease	α-synuclein	α-synuclein
Dementia with Lewy bodies		
Multiple system atrophy		
Fronto-temporal dementia	MAP-τ	MAP-τ
	progranulin	
Amyotrophic lateral sclerosis	superoxide dismutase	superoxide dismutase
Prion diseases	a cellular isoform of prion protein (PrP^c)	a disease (PrP^d) isoform of prion protein

[A26]

Tubulovesicular structures are present in an autopsy case of variant Creutzfeldt-Jakob disease

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Tubulovesicular structures (TVS) are 27- 35 nm in diameter virus-like structures encountered in all transmissible spongiform encephalopathies (TSE) or prion diseases including Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker disease and fatal familial insomnia. Similar particles were also described in tissue cultures infected experimentally with Creutzfeldt-Jakob disease (CJD) agent. Two cases of variant CJD (vCJD) examined as brain biopsies were observed in a single specimen but never in autopsy samples. Here we describe a TVS in an autopsy sample of vCJD. By thin sec-

tion electron microscopy, we observed a cluster of three neuronal processes containing TVS. This report shows that TVS may be found also under suboptimal conditions of brain autopsy and again stresses the search for their true significance.

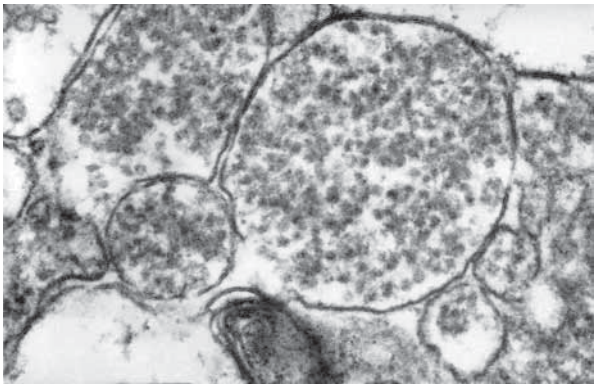

Fig. 1. A cluster of three neuronal processes containing numerous TVS. Orig. magn., ×66 000

[A27]

Antiproliferative effect of tachykinin-opioid hybrid peptide analogues on human glioblastoma T98G cells

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Tachykinins are a group of excitatory neuropeptides that are widely distributed in neuronal and glial tissues of the human central nervous system. The most important member of them is substance P (SP), being a ligand for neurokinin-1 tachykinin receptor (NK1). Substance P and its target are considered responsible for increase in glioma malignancy. Growth regulation of neoplastic cells is also governed by opioid receptors MOR (μ opioid receptor), DOR (δ opioid receptor) and KOR (κ opioid receptor) that constitute the large G protein-coupled receptor (GPCR) family.

Our study has shown that hybrid coupling of NK1 receptor antagonist and opioid receptor agonist sequences has an antiproliferative effect on human glioblastoma T98G cell line documented by decrease in Ki-67 labelling index and limitation of growth rate of glioma cells. Tested compounds also affected cell survival and reduced colony formation. There were no marked alternations in GPCR receptor immunoexpression.

Tachykinin-opioid analogues, which not only diminish cell proliferation but also attenuate cancer-associated pain, seem to give a significant perspective for new therapy development.

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[A28]

Immunohistochemical and ultrastructural studies in differential diagnosis of rhabdoid meningiomas

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We present the morphological pattern of rare, rhabdoid subtype of meningioma. This variant of meningioma corresponds to WHO grade III and is associated with histological features of malignancy and aggressive clinical behaviour determined by local recurrences and/or remote metastases. Its histopathology, resembling other tumours of different histogenesis, might cause some difficulties in correct diagnosis.

Three presented cases of rhabdoid meningiomas contained large tumour cells with vesicular, often eccentric nuclei with distinct nucleoli and abundant cytoplasm containing cytoplasmic eosinophilic inclusions. The central parts of the tumour were composed of necrotic tissue containing well-preserved sheets and clusters of rhabdoid-like cells. The rhabdoid parts of the tumour exhibited high proliferation rate (80% of MIB1-positive cells). In one tumour the classical meningothelial features were evidenced only focally by whorl formation, whereas the other ones were entirely rhab-

doid. Immunohistochemically, the rhabdoid tumour cells were positive for vimentin and S-100 protein and showed focal epithelial membrane antigen and cytokeratin expression. The ultrastructural analysis demonstrated features that confirmed the diagnosis of meningothelial origin, i.e. interdigitating cell processes joined by numerous desmosomes and the presence of distinctive meningocytic cells.

Both immunohistochemical and electron microscopic study were very useful in differential diagnosis and treatment of such rare subtypes of meningiomas.

[A29]

Choroid plexus. Morphology and participation in the clearance of amyloid beta protein

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An inadequate metabolic clearance within the brain or an improper balance of import and export of amyloid beta protein at brain barriers are considered to be critical events in brain amyloidosis and the aetiology of Alzheimer's disease. One of the brain barriers occurs at the choroid plexus (CP) and it separates blood from the cerebrospinal fluid.

This study examined the morphological changes that appear in human CP tissue during the life-span and the immunohistochemical expression of some proteins which participate in the clearance of amyloid beta protein (Aβ).

Brain tissue samples and/or CP removed from the ventricles were obtained post-mortem from patients of various age (from 5 to 82 years). The tissue samples were fixed in formalin and embedded in paraffin and the sections were used for immunohistochemistry to detect low density lipid receptor-related protein (LRP) and receptor for advanced glycation end-products (RAGE). Brain samples were also fixed in 2% glutaraldehyde and embedded in Epon and semithin sections were used to measure the number of epithelial cells containing lipofuscin.

The results of our study showed that already in children over the age of 10, CP had undergone modifications. Progressively with the age of the patients, the number of epithelial cells containing lipid vacuoles and other lipofuscin deposits increased. The epithelial basement membrane (BM) and the connective tissue became more abundant and thicker.

The immunohistochemical expression of LRP and RAGE in CP had undergone parallel age changes. Both proteins were found only in CP epithelial cells where the blood-cerebrospinal fluid barrier (BCB) is localised. In young human subjects expression of RAGE, which is the protein responsible for transport of serum soluble Aβ across the brain barriers, was weak but became stronger in CP of aging patients. The protein was localised beneath the abluminal surface of the epithelium and over the surface which made contact with the BM. LRP as the second protein involved in the metabolic pathways of Aβ in young patients was detected in the cytoplasm of epithelial cells but in aging subjects the strongest LRP expression was localised around lipid vacuoles and lipofuscin deposits.

All our results document that CP plays an important role in the clearance of the brain Aβ during normal aging and that the function of the blood-cerebrospinal fluid barrier modified during the life-span may participate in the accumulation of Aβ within the brain tissue.

[A30]

Different forms of amyloid beta protein deposits in the brain of patients after acute ischaemia (cardiac arrest)

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It was suggested that severe ischaemia may be a risk factor for the accumulation of amyloid beta protein in the brain and may play a role in the pathogenesis of human dementia.

The aim of the present study was to examine the presence and distribution of amyloid beta protein in the brain of patients affected by acute ischaemia caused by cardiac arrest.

The study was performed on the brains of patients who died during four weeks following successful resuscitation. Matched control patients were not resuscitated and died suddenly after cardiac arrest. All patients were divided into four groups depending on the age of life. Brain samples were fixed in formalin and embedded in paraffin. For detection of amyloid beta protein immunohistochemical methods with a battery of primary antibodies generated against various domains of the amyloid beta protein were used. Some brain sections were also stained with cresyl violet, Congo red and Bielschowsky silver methods.

In all resuscitated patients amyloid beta protein was found in perivascular areas of some blood vessels of grey and white matter, and in some subependymal, and submeningeal areas of the brain. Moreover, numerous nerve cells of the neocortex and subcortical nerve centres were strongly immunopositive to amyloid beta protein.

In the oldest (over 65 years of age) group of resuscitated as well as control patients, in addition to the above localization, numerous focal deposits of this protein in the form of diffuse and/or senile plaques were found in the neocortex and hippocampus. Large diffuse deposits of amyloid beta protein were detected in the molecular layer of the cerebellum as well.

The results of our study led to the conclusion that the acute ischaemia led to dysfunction of the brain barriers and caused brain penetration of the serum fluid containing amyloid beta protein. The nerve cells appeared as a second source of amyloid beta protein in the brain of resuscitated patients. However, diffuse and senile plaques found in the brain of the oldest resuscitated and control patients were not caused directly by the acute ischaemia.

[A31]

Skeletal muscle pathology in children with *SCO2* gene mutations

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A product of the *SCO2* gene is involved in the assembly process of mitochondrial cytochrome *c* oxidase (COX). In 2001 we reported our first patient with spinal muscular atrophy-like (SMA-like) muscle lesions and COX deficiency in muscle who then appeared to have homozygous 1541C>A *SCO2* gene mutation. Since then, fourteen new cases with *SCO2* gene mutations have been revealed in our institution.

Clinical course of the disease suggested mitochondrial encephalomyopathy in all patients. Muscle biopsy showed four discernible patterns: A – SMA type I with COX deficit; B – SMA-like with variable COX activity; C – Neurogenic atrophy with COX deficit; D – Only variability of muscle fibre diameter with variable COX activity.

Conclusion: Our observations confirm that 1541C>A mutation in the *SCO2* gene is frequently associated with the neurogenic pattern of skeletal muscle involvement. SCO2 gene mutation should be included in differential diagnosis in children with spinal muscular atrophy and "SMA-like" patterns, as well as dispersed neurogenic atrophy.

[A32]

Does overexpression of SOD-1 gene in the rat model of familial amyotrophic lateral sclerosis lead to changes in nuclear proteins?

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In our previous studies on the animal model of familial amyotrophic lateral sclerosis (fALS), very weak astrocytic reaction and immunoreactivity in asymptomatic stages of the disease in rats with overexpression of mutated SOD-1 gene was found. We concluded that this phenomenon might be related to cytoskeleton damage and tau protein accumulation in astrocytes. More detailed investigations on the same rat fALS model revealed the presence of Alzheimer II cells ("naked nuclei") and enlargement and deformation of astroglial nuclei in anterior horns of the spinal cord. Astrocytic nuclear deformity was observed in animals at the age of 93 days and older. At the level of electron microscopy, in 60-day old rats, astrocyte nuclei were normal but starting from animals at the age of 120 days (early paretic stage) eminent alterations in nuclear size and shape resembling changes observed in laminopathies were found. In the immune reaction to nuclear lamin B, deformation of nuclei was visible in fluorescent microscopy in rats at the age of 93 days (asymptomatic stage) and in the paretic stage. Negative immunoreactivity of astroglia to lamin A/C requires further investigations.

[A33]

BACE1 gene polymorphisms in Polish centenarians

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Amyloid precursor protein (APP) can be cleaved by α- or β- and γ-secretases. β-secretase was identified as transmembrane aspartic protease β-site APP cleaving enzyme 1 (BACE1). BACE1 levels and activity are increased in Alzheimer's disease (AD) brains. BACE1 is crucial for β-amyloid generation in the amyloidogenic pathway, which plays an important role in AD pathogenesis. A few papers have been published showing a possible association of polymorphisms within the *BACE1* gene and the occurrence of Alzheimer's disease. The authors investigated two polymorphisms in the *BACE1* gene in Polish centenarians compared to a group of young healthy individuals showing no signs of cognitive impairment. The percentage of CC genotype at codon 262 in exon 5 in centenarians was higher than in the controls. The percentage of GG genotype in single nucleotide polymorphism in intron 5 in centenarians was slightly lower than in young healthy controls; however, this difference was not statistically significant. Further study is needed to confirm a possible association of the examined polymorphisms with longevity and/or dementia in the elderly.

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[A34]

Ultrastructural, immunohistochemical and confocal study of florid plaques in variant Creutzfeldt-Jakob disease

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Amyloid plaques are a hallmark of transmissible and non-transmissible brain amyloidoses, and in human transmissible spongiform encephalopathies occur in sporadic CJD (sCJD), kuru (both of which may contain kuru plaques), Gerstmann-Sträussler-Scheinker disease (GSS) (multicentric plaques) and variant CJD (vCJD) (florid plaques). The ultrastructure of kuru and multicentric plaques has been studied for decades, but florid plaques in vCJD have been described at the ultrastructural level in only one case report.

To rectify this situation, we studied vCJD plaques systematically and compared them to plaques in kuru, sCJD, GSS and Alzheimer's disease (AD).

Amyloid plaques were studied by transmission electron microscopy and image analysis in 5 cases of variant CJD, 3 cases of GSS, 2 cases of sCJD, 1 case of kuru and 5 cases of AD. Additionally in 1 case of vCJD, 2 cases of GSS, 1 case of kuru and 2 cases of sCJD amyloid plaques were studied by immunohistochemistry and laser confocal microscopy.

Florid plaques were either compact (resembling kuru plaques) or more diffuse; in both forms the radiating fibrils were organized into thick "tongues", in contrast to classical kuru plaques. Florid plaques also contained dystrophic neurites; the latter contained lysosomal electron-dense bodies or vesicles. Microglial cells were found within florid plaques and in certain specimens the amyloid fibrils were clearly present in membrane-bound pockets of microglial cells. In vCJD there was significant MAP-tau immunoreactivity around the plaques. In sCJD, GSS and kuru minimal immunoreactivity of MAP-tau was also observed at the periphery of the plaques.

Overall, the ultrastructure of florid plaques of vCJD is more reminiscent of neuritic plaques in AD than kuru or multicentric plaques. The reasons for these differences in prion protein plaque structures are poorly understood, but may reflect differences both in the strains of the transmissible agents responsible for these disorders and in host factors.

[A35]

Synucleinopathy with features of both multiple system atrophy and dementia with Lewy bodies

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Dementia with Lewy bodies (DLB) and multiple system atrophy (MSA) are two alpha-synucleinopathies regarded as distinct entities. DLB is the second most frequent neurodegenerative dementing disorder in the elderly after Alzheimer's disease. Clinically, it is associated with fluctuating cognition and hallucinations in association with neurological features of Parkinsonism. DLB is neuropathologically characterized by the presence of Lewy bodies (LBs) in the brain. MSA is a sporadic neurodegenerative disease clinically characterised by varying degrees of Parkinsonism, cerebellar ataxia and/or features of autonomic failure. The term MSA was coined to unify three separate entities: striatonigral degeneration, olivopontocerebellar atrophy and the Shy-Drager syndrome, which may overlap clinically and pathologically. However, it was not until 1989, when Papp and Lantos described filamentous inclusions in the cytoplasm of oligodendroglial cells in the brains of patients with MSA cases, irrespective of clinical symptoms, that the existence of MSA as a clinicopathological entity was confirmed. We report here a case of a combined alpha-synucleinopathy affecting both the brainstem in MSA-like fashion and the neocortex in DLB-like manner. We re-examined an archival autopsy case of a 51-year-old woman with progressive cognitive deficits. We used histopathology, immunohistochemistry, electron microscopy and laser confocal microscopy. The brain showed typical

neuropathological features of MSA as well as numerous Lewy bodies in the brain cortex, fulfilling the criteria for neocortical DLB. Another interesting finding was the presence of multiple prominent round or ring-like inclusions in neurons of the dentate fascia, which were readily visible in routine haematoxylin and eosin staining. Immunohistochemically, oligodendroglial and neuronal inclusions as well as Lewy bodies were strongly positive for α-synuclein and ubiquitin. The neuronal inclusions of the dentate fascia also showed strong α-synuclein immunoreactivity, but were negative for anti-microtubule associated protein (MAP)-τ antibody.

Conclusions: Our case suggests that DLB is closely related to MSA and that the synucleinopathies, similarly to tauopathies, should be regarded more as a continuum of changes than as strictly divided entities. Whether cases with massive neuronal changes in dentate fascia are linked to diffuse Lewy bodies in the brain cortex remains to be established.

[A36]

Molecular mechanism of cognitive impairment in brain aging and neurodegenerative disorders. Novel therapeutic strategies

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Our previous study indicated that aging altered glutamatergic receptor nitric oxide (NO)/cGMP signal transduction. In this study the role of NO/cGMP and specific isoforms of cGMP phosphodiesterases (PDE) in aging of the cholinergic system and in cognitive function was investigated. Our data indicated significant decrease of the cGMP level in the cholinergic system of aged brain (24-month old rats) compared to adult (4-month old rats). In situ hybridization study indicated the significant enhancement of gene expression for PDE 2 and 9 and no alteration for PDE 5 in aged brain. Inhibition of PDE 2 and PDE 5 leads to elevation of neuronal isoform of NO synthase activity in the hippocampus of adult animals but in aged only inhibitor of PDE 2 had an effect. Inhibitors of both PDE 2 and 5 (Bay 60-7550 and zaprinast respectively) significantly improved memory in adults and Bay 60-7550 had a positive effect on cognitive function in aged animals. Our data suggested that enhanced influx of Ca2+ ions through cGMP regulated channels influenced the pool of nNOS activity involved in memory. However, NO liberated in excess by amyloid beta (AB) peptides in an Alzheimer's disease (AD) animal model and in inflammation is toxic. It leads to activation of cytosolic phospholipase A2 (cPLA2), arachidonic acid metabolism by cyclooxygenases (COX) and lypoxygenases (LOX). The nuclear target for NO and PLA2-mediated signalling, poly(ADP-ribose) polymerase (PARP-1) is also stimulated under these conditions. Our results indicated that AB1-42 and lipopolysaccharide (LPS) evoked inflammation-enhanced COX-2 and 12-LOX expression and activity and PARP-1 activity and decreased object recognition, locomotion and exploration in mice. COX 2, 12-LOX and PARP-1 inhibitors protected mice against memory deficit and locomotor disturbances. These results indicated that inhibitors of cGMP-PDE are efficient in memory improvement in adult and aged brain; however, in neurodegenerative disorders the suppression of gene expression of both isoforms of COX and 12-LOX and inhibition of PARP-1 activity should offer an effective protective strategy.

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[A37]

Biochemical analysis of human brain gliomas using techniques based on synchrotron radiation

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To better understand the biochemical mechanisms that may participate in the pathogenesis of brain gliomas an elemental and molecular investigation was performed

using techniques based on synchrotron radiation. For this purpose, synchrotron radiation X-ray fluorescence (SRXRF), X-ray absorption near edge structure (XANES) spectroscopy and Fourier transform infrared microspectroscopy (FTIR) were applied. The SRXRF technique was used for topographic and quantitative elemental analysis of thin tissue samples. For the same samples mapping of the different forms of sulphur and iron was performed with XANES spectroscopy. The investigation of changes in the main biological molecules (carbohydrates, lipids, proteins, nucleic acids) in the case of brain gliomas was performed using FTIR microspectroscopy.

Tissue samples were taken intraoperatively from patients with different grades of brain gliomas. The specimens were frozen and cut into sections of 20 or 10 micrometers thick in a cryo-microtome. The slices were fixed onto Ultralene foil and frozen.

Two-dimensional maps of selected element distribution were determined. The preliminary research showed that for all cases the elements P, S, Cl, K, Ca, Fe, Cu, Zn, Br and Rb were present in human brain glioma samples. Additionally, in selected samples the elements Hg, La, Ni, Ru and Gd (the result of applying Gd-containing magnetic resonance imaging agent) were detected. It was observed that the levels of S, K and Cl are higher in glioma cells than in surrounding tissue. The masses per unit area of elements were calculated for the glioma cells and surrounding areas.

The analysis of iron oxidation states showed that Fe occurs in the glioma tissue mainly in the oxidized form (Fe^{3+}). Moreover, low intensity of pre-edge peak indicates that Fe occurs mainly in the complexes of octahedral geometry, in which the number of ligands bound to the transition metal ion is 6. Additionally it was noticed that cancer cells accumulate sulphur mainly as sulphide (S^{2-}) form. The preliminary results indicated also higher accumulation of this form of sulphur in glioma of grade IV of malignancy in comparison with the samples of grade II neoplasms. The presence of sulphate (S^{+6}) species was revealed in histological structures outside the cancer cells.

The elemental analysis was coupled with determination of main biomolecules of the samples. The tissue areas were mapped to generate two-dimensional images of the molecules of interest. The major spectral differences between control and cancerous tissues were identified for the vibrational frequencies characteristic for proteins and lipids.

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[A38]

Wilson's disease with disseminated intravascular coagulation (DIC)

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Wilson's disease (WD) is an autosomal recessive inherited disorder of copper metabolism, transport and storage resulting in the accumulation of copper in various organs including the liver, cornea and brain. Memory disturbances in the neurological type of the disease can progress to cognitive impairments (subcortical dementia).

In our presented patient, a 31-year-old woman, the first manifestations of Wilson's disease were deterioration in memory, concentration problems and progressive movement disorders. The diagnosis of Wilson's disease was confirmed at 30 years of age, by genetic analysis, which revealed presence of 2 H1069Q mutations in both alleles of APTP7B (a copper transporting P-type ATPase), at which time she started penicillamine treatment without notable improvement in her memory and in her motor signs. Clinical and laboratory hepatic damage signs were not observed at that time. The family history revealed that her 2 sisters died with the symptoms of fulminant liver failure at the ages of 18 and 17 (most probably these were cases of Wilson's disease). In our patient, after 1.5 months in spite of continuation of the treatment, the disease progressed rapidly and the patient died of fulminant thrombocytopenia, bleeding complications and renal infection.

Autopsy revealed macronodular liver cirrhosis and extensive multifocal microthrombi and bleeding in the heart, lung, kidneys and suprarenal glands. Immunohistochemical study of oedematous brain showed numerous Opalski and Alzheimer's I cells and comparatively few Alzheimer's II cells localised in all grey and white

matter structures. The focal spongy and swelling tissue changes were predominant in the basal ganglia. Additional central pontine myelinolysis with demyelination of the basis pontis was found. In all studied structures, occlusive microthrombi characteristic for disseminated intravascular coagulation were observed.

It is suspected that the coagulopathic changes presented in this case (activation of the coagulation cascade with thrombocytopenia and DIC in the brain and bleeding in the internal organs) are a sign of fulminant hepatic failure leading to decompensation of probably previously silent liver cirrhosis.

[A39]

Polymorphisms of genes *MTHFR* and *MTR* and level of thiols in patients with epilepsy treated with antiepileptic drugs

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Antiepileptic drugs (AEDs) in patients with epilepsy may increase the concentration of homocysteine (Hcy), and alter the metabolism of thiols. Homocysteine is a sulphur amino acid which is degraded in the human body in two main metabolic pathways: sulphuration and remethylation. In remethylation Hcy is converted to methionine (Met). Methionine may be remethylated to homocysteine via SAM (S-adenosylmethionine) and SAH (S-adenosylhomocysteine). SAM is the main donor of methyl groups in many metabolic processes. Hcy may undergo remethylation due to MTHFR and MTR proteins. Hcy is a known risk factor of vascular diseases.

The aim of the study was to determine the incidence and genotype frequencies of C677T polymorphisms of *MTHFR*, and A2756G polymorphism of *MTR*, and estimate the plasma levels of Hcy and Met in patients with epilepsy, treated with valproic acid (VPA), carbamazepine (CBZ) and new generation antiepileptic drugs. Results of analysis of thiols were compared with the genotypes of the polymorphisms of genes involved in Hcy metabolism.

Studies were conducted on plasma levels of Hcy and Met, estimated by HPLC with electrochemical detection in 55 patients with epilepsy treatment AEDs, at age 19-65 years, and 61 individuals at age 22-65 years, as a control group, as well as estimation, by restriction analysis, of frequency of the following gene polymorphisms: *MTHFR* (C677T) and *MTR* (A2756G). The studies disclosed that the level of Hcy increased higher than 15 μM in 19 patients (34%) taking AEDs. Moreover, in patients treated with AEDs the level of Hcy was higher ($p<0.01$), but the levels of Met ($p<0.01$) and Met:Hcy ratio ($p<0.001$) were lower than in the control group. In patients treated with CBZ it significantly decreased the levels of Met and Met:Hcy ratio as compared to patients treated with VPA. In patients with epilepsy with frequency CT, *MTHFR*, C677T and AG, *MTR*, A2756G there were significant differences of the level of Hcy ($p<0.05$) and Met:Hcy ($p<0.01$, CT, *MTHFR*, C677T and $p<0.05$, CT, *MTHFR*, C677T), and in patients with epilepsy with frequency AG, *MTR*, A2756G there were also significant differences of the level of Met ($p<0.05$) as compared to the control group.

Monitoring of Hcy levels in patients with epilepsy taking AEDs may be a new diagnostic factor.

[A40]

Angiomatous paraganglioma of the cauda equina manifesting as syndrome of increased intracranial pressure

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Cauda equina paraganglioma (CEP) is a rare tumour, accounting for about 3.5-3.8% of all neoplasms in this region. Clinical symptoms produced by paragangliomas are non-specific and include features of the cauda equine syndrome. Intracranial hypertension as a clinical manifestation of CEP is extremely rarely observed; it has been reported only in 6 cases.

We report a case of CEP in a 61-year-old man who presented with headache and papilloedema. MRI of the brain demonstrated enlarged ventricles but otherwise

failed to disclose any intracranial lesions. Cerebrospinal fluid examination showed very high protein level, 2300 mg/dL, and low cytosis 2. Spinal MRI revealed a well demarcated intraspinal tumour at L3/4 level, with appearance suggesting meningioma. Laminectomy of L3 and L4 was performed and the tumour, which was attached to a nerve root of the cauda equina, was totally removed. Histopathological study of the tumour revealed cellular and immunohistochemical features of paraganglioma with characteristic "Zellballen" pattern and positive reaction for synaptophysin, chromogranin A and cytokeratin of the chief tumour cells as well as with S-100 protein reactivity of the sustentacular cells. In addition, an unusual finding of abnormal angiomatous component of tumour associated with haemorrhagic and proteinaceous fluid extravasation into the tumour tissue and subarachnoid space was observed.

Angiomatous pattern of CEP in the reported case supports the pathogenic role of vascular factors in the complex mechanism of increased intracranial pressure development in this intraspinal tumour.

[A41]

Effect of selected neuroprotective compounds on MNs degeneration in a model of glutamate excitotoxicity *in vitro*

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We determined the effect of selected neuroprotective compounds on neuronal changes in a model of slow glutamate excitotoxicity *in vitro*. The study was performed on organotypic cultures of the rat lumbar spinal cord subjected to glutamate uptake blocker, DL-threo-beta-hydroxyaspartate (THA). It has been documented that chronic THA exposure induced various types of morphological changes including necrosis, apoptosis and autophagocytosis.

In this study the potential neuroprotective effects on THA-induced MNs neurotoxicity was investigated for cytidine-5V-diphosphocholine (CDP-choline), geranylgeranylacetone (GGA) and erythropoietin (ER). Well-differentiated organotypic cultures of the rat lumbar spinal cord were pretreated with one of these neuroprotective agents, i.e. 100 μM of CDP-choline, 10μM of GGA or 5U/ml of ER, and then were subjected to 100μM of THA. The ultrastructural examinations of various experimental groups were performed in JEOL 1200EX on days 3, 5, 9 and 14 post incubation.

The results of this study revealed that pretreatment with CDP-choline inhibited the development of late apoptotic neuronal changes, whereas the early necrotic THA-induced injury as well as autophagic degeneration of MNs was not reduced. In the cultures exposed to GGA + THA, there was not observed any beneficial effect of GGA on THA-induced early necrotic and late apoptotic or autophagic motoneuronal degeneration. Cultures exposed to ER + THA showed inhibition of MNs degeneration, including various modes of degenerative changes caused by THA.

[A42]

Neuropathological manifestation in adult form of type IV glycogenosis

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Glycogenosis type IV is caused by a deficiency of a branching enzyme – α 1,4 glucan 6-transglucosylase. The deficiency results from mutations in the GBE1 gene located on human chromosome 3p12. The neuropathological hallmark of adult form of this storage disease in the CNS is represented by adult polyglucosan body disease (APBD). In the case of APBD the storage material is mainly present in the central and peripheral nervous system.

We analysed a case of a 45-year old unconscious woman who died on the third day after admittance to the hospital. She had suffered from headache, nausea and vomiting in the last month. She had lost her job one year before. Cerebrospinal fluid pressure was increased, with 350 mg% of protein, and 105 mg% of glucose. In

this report we present histological, immunohistochemical and ultrastructural examination of the brain.

Neuropathological examination revealed massive accumulation of polyglucosan bodies (PBs) in the cortex and white matter of the whole brain. These bodies were found mostly in the white matter, around vessels and beneath the pia. The PBs were located in the processes of neurons, astrocytes and microglial cells and generally resembled round or elongated spheres. The storage material in the cytoplasm of neurons and glial cells was visible as fine granules.

Ultrastructurally, PBs consisted of non-membrane-bound deposits of branched and densely packed filaments measuring about 7-10 nm in diameter, typical of polyglucosan bodies. The filaments were mixed with fine granular and amorphous material and small vesicles. PBs usually were large ellipsoid structures but sometimes irregular fibre masses were also visible, particularly in astrocytes. PBs were situated both in the cytoplasm and processes of astrocytes in the vicinity of the remnants of gliofilament bundles. Numerous large PBs located in distended axons caused disruption of their sheaths.

The microglial and oligodendroglial cells showed cytoplasmic and nuclear condensation resembling apoptotic changes. Neuronal cytoplasm was rich in organelles abnormally closely packed including numerous mitochondria with short, concentrated cristae.