ON THE MOLECULAR CORRELATIONS IN THE PATHOBIOCHEMISTRY OF ALZHEIMER DISEASE

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ABSTRACT

The present work describes the major theories of the molecular pathogenesis of Alzheimer's disease and some other dementias. It is very actual nowadays the priogenesis theory. S. B. Prusiner has shown the pathogenic form of molecular configuration of the proteins, he named prions. We discuss the infectious (transmissible), as well as the genetic and especially spontaneous (sporadic) form of their expression; the folding problem and possible therapeutic intervation in nucleation of prions, using sulfated glucosaminoglycans. Interesing data is summarized about the tau-hypothesis. The subcellular mechanisms of kinases interactions in the hyperphosphorylation of microtubule-associated proteins into paired hellical filaments amyloidogenesis are presented in the context of the Nivalin and lithium treatment of Alzheimer's disease. It is still important D. Harmer's free-radical theory. We defend the thesis that the prooxydant - antioxydant balance disturbance may lead ti cell lesions that could explain a part of the neurodegenerations. The theory that defends the role of supraphysiological level of microelements, such as aluminium, cummulation is considered too. The toxic theory interferes with the free-radical and acute phase proteins participance in the pathological activation of the neuroglia theories both. The results of R. Clark at Al. about the association between folate, vitamin B12 and the total serum homocysteine with Alzheimer's disease are marked. In conclusion we offer ethiologically convergent axes of the molecular pathology of Alzheimer's disease: Abnormal protein cummulation; free-radical interactions and immunological patho-phenomenology.

Introduction

The problem of the pathological aging of the brain is got increasing clinical-bio-social importance.

The number of the registered deviations of the clinical dynamics of the neurodegenarations is soaring. Dementias from the group of Alzheimer disease with dramatic clinical progression, that the modern doctor is not prepared to face, are reminding about themselves. The epidemiological incidence of the registered cases is worrying because of the fears of the phenomenon of the medicosocial "iceberg". Considering these facts, the question of the therapeutic approach to these groups of diseases seems incredibly important. No doubt that the building of such approach is practically impossible without knowledge of the discreet pathogenic mechanisms, determining the illness processes. This work is dedicated exactly on these pathogenetic mechanisms, and most of all on the leading moments in the current molecular theories.

1.The theory of prionogenesis

In 1982 S.B. Prusiner called proteinacious infectious particles the discovered in 1968 from Gaydusek proteinacious particles.

They are protein agents, inherent to the plasmalem of the neuron and other cellular populations, as the B-lymphocytes, whose physiological role is not determined yet. It has been proven that they are the basis of

the so-called spongiform encephalopathies: M.Creuzfeldt-Jacob, kuru, scrapie, the fatal familial insomnia, M. Gerstmann – Straussler- Scheinker (11).

In 1992 were hystochemically carted (25) the prion proteins in the brain, and later Prusiner discovered that the prions have two isoforms with identical amino acid consequence (primary structure by Lindenströhm-Lang), but with different secondary organization. It was postulated that there are three variations of the prions – genetic (hereditary), transmissive and sporadic (spontaneous). The genetic mutation of the syndrome of Gerstman – Straussler – Scheinker was isolated. It was the end of initial theory of Gaydusek and Prusiner, who accepted the prion pathology only as necessary infectious. The spontaneous form was considered especially interesting and was potentially connected with the Alzheimer disease. So the interest of the researchers was focused on the post-translation changes of the molecule of the cellular (normal) Pr Pc protein. At first were discovered defects in the structure of the RNA for the amyloid precursor peptide and Pr Pc in the polyzomes of rats. Pr Pc and Pr Psc have the same amino acid consequence, but are different in their three dimentional configuration. The mutant Pr Psc had higher content of beta-sheet and lower content of alpha-spiral structure compared with PrPc (30).

Lundberg and his associates (15) proved that the process of the transformation of the cellular form in the pathogenic one (Pr Psc – from scrapie – a zoonose of the sheep) is actually transformation of the alpha-spiral zones of the PrPc to the beta-sheet rich structures. This lead to the question of the sub-molecular abnormality, that causes the abnormal transition, that changes the tertiary structure of the protein. It is the reason for the amylodogenetic connection and conglomeration. The question was solved recently by Orpiszewski and Benson (19), who proved that the critical moment in the formation of the amyloid fibers is chemical transformation of the aspartyl amino acid into succinimidic and isoaspartyl metyl esters residues in the hydrophobic nucleus of the protein. This transformation is assosiated with the ageing. The authors bring forward the hypothesis, that in the basis of the described process may be is the spontaneous cycling of the asprtyl residues in the hydrophobic nucleus of the prion molecule, named ***nucleation***. So, the nucleation of PrPc shows the way for solving the problem with the tertiary structural changes of PrPc, known as *folding – problem* (the folding into beta-sheet of the poly-peptid chain). And something more – it gave directions to the theraupetic intervention.

According to the discovery of Mellhorn, De Armond and Prusiner, the monoclonal anti-bodies don't attack the prion protein, because its primary amino acid consequence is normal (30). That means that there is no epitop against which to react for the anti-bodies and predictes some difficulties in the therapy. Tatzeldt et. al (24) discovered, that the chemical "chaperones" (low molecular weight attendants) are included in Pr P (prion protein) pathology as protectors of PrPsc. The fact that the mechanism of conversion between the prion molecules is autocatalitic suggested that a synthetic inhibitor couldn't supress the begining of the polymerization. It has been determined that the carbon terminal consequence of the prion is responsible for the subcellular traffic and the transformation of the molecule. The nitrogen one, on the other hand, is responsible for the distribution of the pathogen. The hopes were focused towards this eventual locus minoris resistentiae, and it couldn't be explained solely by the isolated by Tatzeld chemical attendants and the stability of the beta-list rich Pr Psc isoform.

In 1992 was proven as well the existence of ubiquitin (1) – the "messenger of death", that plays role in the recycling of the cellular proteins, in axons, which are not

damaged from the PrPsc plaques of the disease of Crezfeldt-Jacob. This is an argument against the hypothesis for the apoptotic nature of this degeneration.

Recently the extraneural localization of PrP in the *filla olfactoria and tonsiles* was reported (7), that occurs to be of great diagnostic significance.

Together with the prevailing skepticism in the literature (13), there came the publication of Perez and associates (20) who announced, that the sulfated poly-sugars as heparin, ceratan and hondroitin inhibit the neuro-toxicity of the formed PrP fragments amyloid fibers. It comes to show that probably they are involved in the primary act of nucleation, unlocking the polymerization of PrP peptide to fibrils.

2.Hypothesis of the Tau-protein

In difference with the prion theory, this hypothesis is related primarily to the Alzheimer disease, and in less degree with Pick disease and the post-encephalitic Parkinsonism. The tau-protein is microtubular associated protein, which combines with the tubuline of the citoskeleton (by the means of 18 amino acids, attached to the carbon end of the beta-tubulin). The homo and hetero-dimerical polymerization of the tau-protein is a key stage of the amyloidogenesis of the Alzheimer degeneration (6). It had been proven that the products of the coupling of tau - ***paired helical filaments (PHF)*** are result from processes of hyper-phosphorylation and oxydation and their main phosphate acceptory site in the poly-peptide chain of tau is R-3 cystein. The same circumstance leads us to the role of the disturbed enzymatic regulation of the kinase - systems in the neuron. Utton, Van de Candelere, Wagner (27) proved that the phosphorylation of tau from the glycogen-synthetase – 3 beta is a pace-making stage as it comes to the assembling of the microtubules. It has been found that the nitogen-activated proteinkinases, as well as stress-activated c-jun-N-terminal kinase also phosphorylate tau.

As the micro-tubules build peculiar "railways" of the axonal antegrade and retrograde traffic, the *self-assembly* of these "rails" will disturb cardinally the functions of the synapses, as the communication with the soma of the neuron has been interrupted. So, the accumulated PHF and beta-amiloid lead to pathogenetically irreversible *cholynergic dysfunction by the suppression of the acetylcholine synthesis.* (9, 22) (**Fig. 1**)

This pathological link for a long time has been an object of therapeutic attacks (32). Unfortunately, the clinical effect of the anti-cholinesterase instruments, intervening in this link of the type of Nivaline (Galantamine)(Donepezil and Aricept) is far from our expectations for radical solution of the theurapeutic problem of the Alzheimer disease. In a lot of cases its effect is only palliative. It's why Munoz-Montano, Moreno (17) presented method for healing Alzheimer disease with lithium. They reported that the lithium inhibits glycogen-synthase-kinaze-3 and provokes dephosphorylation in the neuronal locuses in vivo, recognized by anti-bodies tau-1 and PHF-1.

Together with these hopeful results there have been coolected data for worrying connection between tau and lipid metabolism. The correlation between epsylon-alel of Apo E4 (enzyme activities of the apolipoprotein are already known!) and the high amount of cases of the family form of the Alzheimer disease has been proven, as well as the expression of the nascent form of Apo E4 izoform of beta-amyloid peptide (31).

The participation of the lipids found its logical explanation when it has been shown that the free (unestrerised) fat acids and especially the minimal concentration of arachidonic acid stimulate the polymerization of tau and amyloid beta-protein. The described processes build serious connection between the primary "idiopathic" and vascular dementias, especially in some

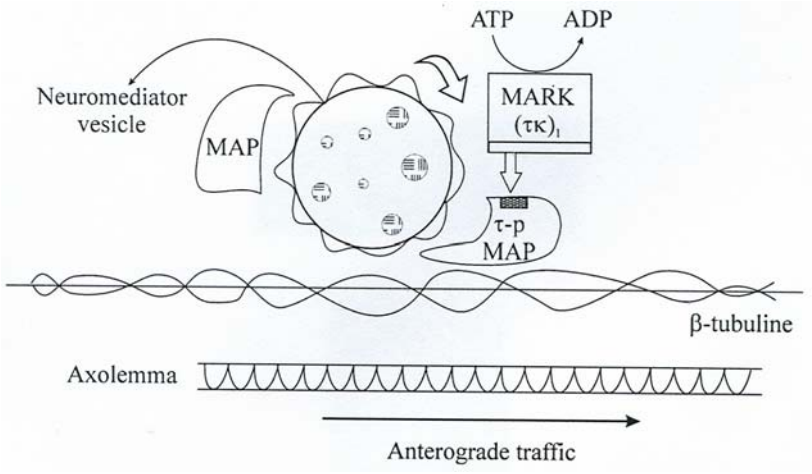

Fig. 1. A schematic illustration of the pathobiochemocal substrate of the tau-hypothesis. MAP is microtubule-associated protein, actually tau; MAPK is the tau-kinase. It provides the chemicl energy for the axonal transport through phosphorylation by ATP (adenosine three-phopsphate), (Illustration by the author).

common aspects of the precipitation of the senile plaques.

3.The theory of free radicals

The reactive metabolites of the oxygen are acquiring high popularity recently. The author of the theory of the connection between the free radicals and ageing D. Harman (8) writes about mutations in the mitochondrial DNA. He believes that in result of this mutation and the consequent disruption in the oxydative phosphorylation, the neuron can't use the oxygen in accordance of its energy needs. After the accumulation of critical mass of free radicals and the consequent lesions, together with the decrease of the ATP-production under the biological need of the cell for vita minima, the clinical manifestation of the disease can be expected. The mentioned above metacatalizated oxydative damage of the amyloidogenic proteins – beta-amyloid and tau, can be assumed (10).

Here is the place to mention that the *Al+ simplifies and induces the toxycity of the iron ions,* which participate in the catalization of reactions, which products are active variations of the oxygen (**reactions of Fenton**).

The role of the free radicals in the pathogenetics of Parkinsson Disease deserves special attention. The idea that what's happening there is oxydative death of the neurons of the nigra substance, which leads to increased activitiy of the mono-amino-oxydase-B, is supported ex juvantibus by the positive effect of the system treatment with MAO-B inhibitor Jumex (Selegilin). (32). The positive effect of the same product on affected with Alzheimer disease gives place to the thought of the role of the free radical theory in the puzzle of the molecular pathogenesis of the dementias in general. We know that the late Parkinsson Disease often manifests itself with dementia. And as the ischemia and atherosclerotic-hypertensive conditions are often connected in a great degree with the oxydative stress, this also adds the vascular dementias to the group of the neuro-degenerations, where the free radicals have significant etiopathogenetic participation.

T. Thomas (26) believes that the beta-amyloid reacts through its interaction with endothelium, stimulating the blood cells for excessive production of superoxide-anion. The same cooperates for the intensification

of the vessel alterate changes.

4.The aluminum hypothesis

It has been described in the works of Dyrks (3). Acoording to him, the aluminum facilitates the release of iron from feritine and in this way accelerates the lipid peroxydation of the biomembranes, or, with other words, acts as prooxydant. It has been assumed that there exist some mechanisms for direct cellular injury (16). It needs to be underlined that some authors (12) consider the aluminum in its role as risk factor on populational level. It is believed that other microelements could increase the effect of oxyde toxic noxes.

5.The role of the acute-phase proteins and the pathologically activated glia

In her neuromorphological studies D. D. Orlovskaya (18) notes the extreme level of fiber astrocytes and increased filaments, fibrilar acid protein in the nerve tissue of patients with presenile dementia from the type of Alzheimer's disease. She also discovered that the glial proliferative fibers surround the lipid centers in combination with sharp decreasing of the levels of the brain creatin-kinase, which points towards deeply injured metabolism and regulation in the energy exchange, which accompanies the obvious existence of specific form of neuroinflamation.

The works of D. Orlovskaya raised the question about the immunological ambivalency of the neurodegenerations. On one hand, the autoagressive reactivity of the glia is pathologically increased against the myeline-basic protein (registered in the cases of multiple sclerosis), and so is the immunobiological tolerance against the prions identified as primarily self-molecules. At the same time it has been proven that the acute-phase proteins are interfering in the laboratory profile of Alzheimer Diseases, which lead to the infectious-alergic component of the illness. Licastro (14) and associates, using the ELISA method found high levels of liquor alpha-1-antitrypsine.

It is known that the *alpha 1- antitrypsine and alpha 2-macroglobuline* belong to the group of serine **protease inhibitors (serpines),** which have modulating effect over the plasma proteolytic systems, involved in the process of inflamation. Using ELISA, Wood and associates (28) demonstrate increased levels interleukins 1 and 6 and significant increase of alpha2-macroglobuline, indicated as IL-6, as cellular mediator of the immune response. Interesting results are submitted by Ganter, Strauss and associates (5). On the basis of the fact that the alpha 2-macroglobuline suppresses the secretion in a higher degree than the synthesis of *beta-amyloid-percursor protein (APP)* they conclude that the alpha2-macroglobuline is obviously an inhibitor of the process of the maturation of the APP. A brief prewiew of the immunopathochemical interactions of the neuroglia is presented on the **Fig. 2**.

The alteration of the ganglial cells leads to extraordinary release of glutamate, a physiological excitatory neurotransmiter. It cumulates in toxic concentrations in the brain and potentiates the degenaritive process, binding to its usual receptor sites - the NMDA and AMPA.The overstymulation of NMDAr is known as excytoxycity.Thus was the NMDA receptors antagonists (Memantine) treatment in the multiconceptual treatment of Alzheimer Disease proposed (21, 23, 29).

Again Wood and associates supposed the regulatory relations between the temporal cortex and the neuro-immune system of the cytokines. Other data related to the increased levels of the C-reactive protein and interleukin-6 antagonist receptors, together with the mentioned above raise more questions than give answers. There is no doubt, however, about the involvement of the immune system, in one way or another, into the pathogenesis of the Alzheimer disease.

R. Clarke (2) and associates recently

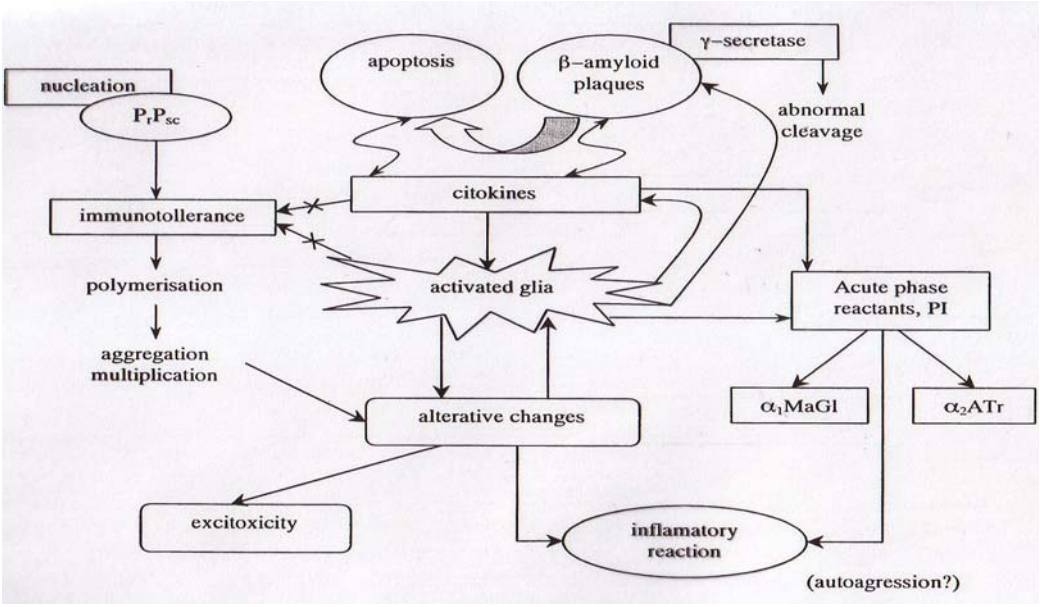

Fig. 2. Action of neuroglia in the disturbance of the the local immunological homeostasis in neurodegenerative diseases, (Illustration by the author).

have shown statistically significant connection between the folate, vitamin B12 and the total serum homocystein with the Alzheimer's disease – facts, whose fundamental explanation is a matter of the future.

Conclusions

My theoretical conclusion is that the molecular pathogenesis of the Alzheimer disease has three basic dimensions, which converge towards one common pathophenomenology – the cellular death.

1. Accumulation of abnormal quantity of proteins with amyloidogenic nature – β-amyloid, Pr Psc, tau, presenilin, with modified structure and normal content.

This is the reason for the intra- and extracellular "blocking" of the neuron, loss of its specific functions and death (4).

2. The lipid peroxydation by the free radicals. This is a process which reflects on several stages of the pathogenesis. As an additional factor is involved the over-physiological accumulation of aluminum.

The immunobiological pathophenomenology: inadequate relations of the neuroganglial cells and glia and the involvement of the acute-phase proteins in the induction of the injury.

As I am foreign to he constitutional determination of the etiology, I assume that the genetic particulars are only a basis for action for the unlocking noxes (33).

The conducted review from the study of the molecular pathogenesis of the Alzheimer disease leads me to the conclusion that the fundamental-empiric quantitative accumulation in the sphere of our knowledge about the ageing brain is far from over, and what we've gathered so far still hasn't reached its synthesized and cognitive gnostic and diagnostic form, in which it can be theoretically and practically useful.

Without claiming for explicity, the presented review is aiming at casting some light on a more actual approach in the search of the causal and pathogenetic treatment of the Alzheimer's disease – an approach, build upon the conception of the

molecular medicine. We are convinced that the modern clinicist must routinely use in his work this unpopular yet scientific-cognitive instrument and so get closer to the impeccability, which is required by the new millennia.

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