Interaction of hemoglobin and haptoglobin with Aβ peptides and amyloid fibrils

Reakcja hemoglobiny i haptoglobiny z peptydami Aβ i fibrylami amyloidowymi

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Summary

Human degenerative diseases are associated with the deposition of proteinaceous fibrils or plaques commonly known as amyloid. Among proteins reacting with Aβ are hemoglobin (Hb) and acute phase protein - haptoglobin (Hp). Formation of the Hp-Hb complex inhibits Hb-mediated generation of lipid peroxides and hydroxyl radicals during inflammation. Some other signaling molecules include neurosecretaries, family of metalloproteinases of the ADAM's family – Alzheimer associated Aβ promote amyloidogenesis in Alzheimer's disease (AD). Alzheimer associated protein (ALZAS) and neural thread protein (NTP) – is a brain protein associated with the pathological changes in AD – brings about early neuronal cell death. Urine NTP sometimes fulfills several criteria for an”ideal biomarker”. Markers typically used for non-AD dementive disorders include 14-3-3 proteins, neuron specific enolase, S-100 protein, heart-type fatty acid-binding protein (H-FABP). Changes in the functions of immunological system are common in AD and healthy elderly subjects – these are CD8, CD28, CD71, in inflammation IL-16, IL-18, IL-1 superfamily. TGF-β promotes amyloidogenesis in AD. Proinflammatory TNF-α may take part in pathogenesis of AD. The use of nanobiotechnology, combination of amyloid-specific monoclonal antibodies with an ultrasensitive nanoparticle-based protein makes that present laboratory diagnostics of AD applies proteomics, metabolomics and pharmacogenomics techniques.

Streszczenie

Wśród białek reagujących z Aβ, przyczyniającymi się do rozwoju choroby Alzheimera są hemoglobina (Hb) i haptoglobina (Hp) – białko ostrej fazy. Kompleks Hp-Hb hamuje powstawanie nadtetlenków lipidowych i rodników hydroksylowych w stanach zapalnych. Do związków “sygnalowych” należą neurosekretazy, metaloproteinazy rodziny ADAM – Alzheimer Associated Protein (ALZAS) i Białko Nici Neuronalnej (NTP). Do białek oznaczanych w chorobach niealzheimerowskich należą białka 14-3-3, swoista enolaza neuronowa, białko S-100, białko występujące w sercu, wiątce kwasy tłuszczowe. ALZAS w surowicy stanowi użyteczny a zarazem prawdozborny marker diagnostyczny AD. NTP - związany jest ze zmianami patologicznymi w AD. Oznaczany w moczu wprawdzie spełnia kryteria „doskonałego biomarkera w AD, jednak należy podejść do tego z pewną dozą krytycyzmu. Zmiany w układzie immunologicznym występujące w AD i u osób starszych – dotyczą CD88, CD28, CD71, w stanach zapalnych IL-16, IL-18, naddrodziną IL-1. TGF-β promuje amyloidogenzę w AD. Z kolei TNF-α – cytokina prozapalna – może uczestniczyć w patogenezie AD. Stosowanie nanobiologii, połączenie specyficznych przeciwiał β-amyloidowych ze strategią oznaczania białek, polegającą na równoczesnym użyciu stężeń attomolarnych sprawia, że współczesna diagnostyka laboratoryjna AD opiera się na proteomice, metabolomice i farmakogenetyce.

Key words: hemoglobin, haptoglobin, Aβ peptides, amyloid fibrils

Słowa kluczowe: hemoglobina, haptoglobin, peptydy Aβ, fibryle amyloidowe

The pathology of more than 40 human degenerative diseases is associated with the deposition of proteinaceous fibrils or plaques commonly known as amyloid. These “protein deposition diseases” affect many tissues and organs of the human body and are comprised of various sporadic and occasionally familial amyloidogenic disorders, e.g. Alzheimer’s (AD) and Parkinson’s (PD) diseases, a number of transmissible prion-based disorders (e.g.Creutzfeld-Jakob disease) and non-neuropathic disorders such as type 2 diabetes and systemic lysozyme amyloidosis [18]. Among the elements of metabolic degeneration in AD biomarkers play a special role. Multiple uses of AD biomarkers allow less invasive, more accurate diagnosis, response to treatment, identification of subjects with greater risk of developing AD, also in cases of
so called “preclinical” disease [6].
Proteins that interact with Aβ-peptides may activate its de-
position or pathogenicity, and thus contribute to the devel-
opment of AD [16]. Among specific proteins with a strong
affinity for Aβ the α chain of hemoglobin (Hb) derived from
rat brain homogenates was identified. Hb co-immunopreci-
ipated with Aβ from both AD brain tissue and plasma. Ab-
normal levels of Hb and heme have been associated with
brain and vascular tissue in AD. Hb-derived peptides and
Hb mRNA levels are increased in AD brains. Brain Hb levels
in AD were the highest in the hippocampus and parietal gray
and white matter and the lowest in the cerebellum, and there
was co-localisation of Hb with senile plaques and cerebral
amyloid angiopathy. The increased presence of Hb and its
break products in the brain probably derives from circula-
ting erythrocytes as a result of endothelial and the blood
brain barrier injury with subsequent leakage of plasma or
blood components into the perivascular space, where addi-
tional heme iron-mediated damage may occur. Hb contained
in red blood cells is normally retained in the reduced state
(oxyHb:Fe2+). Free Hb undergoes spontaneous oxidation to
methemoglobin (methHb, Fe3+). These actions could account
for some of the vascular pathology and neuronal injury or
death in AD. Additionally, competition for free Hb, outside
of the normal haptoglobin and related scavenging systems,
may permit or enhance vascular injury [15].
Haptoglobin (Hp) is a α2 secreted acidic glycoprotein pro-
duced mainly in the liver and found in most body fluids (se-
rum, saliva, amniotic fluid, ascites etc) of humans and other
mammals. Essentially three major forms of human Hp, des-
ignated Hp 1-1, Hp 2-2 and Hp 2-1 are due to two alleles
HP1 and HP2. The mature Hp is a glycoprotein that contains
16% of carbohydrates (N-acetylglucosamine, mannose, ga-
lactose, fucose and sialic acid) [1, 4, 5, 7]. The levels of Hp
in human plasma are increased up to 8-fold during various
stresses (e.g. inflammation) leading to be named as an acute
phase protein (APP). Proteomics approaches to analyze ce-
rebrospinal fluid (CSF) samples derived from AD patients
demonstrated a gradual decrease or absent Hp precursor
allele 1 and plasma retinol-binding protein from patients with
mild cognitive impairment (MCI) and AD compared with the
age-matched normal subjects. Expression levels of the both
markers were very high in normal subjects and were very
much decreased in the MCI group. This may show the pro-
gression from normal to MCI and subsequently to AD [12].
Hp binds rapidly and irreversibly with hemoglobin (Hb).
The binding is one of the strongest known non-covalent interac-
tions in biology, the association constant being greater than
10-15 mol/l. Formation of the Hp-Hb complex inhibits Hb-
mediated generation of lipid peroxides and hydroxyl radia-
cals that occur in areas of inflammation. Hp has also been
implicated in immune regulation. Hp can potentially inhibit
the in vitro formation of amyloid fibrils under physiologically
relevant conditions, retaining the ability to inhibit amyloid for-
mation even when complexed with Hb. Hp exerts its effect
on amyloid formation primarily by interacting with species on
the amyloid-forming pathway prior to fibril elongation early
interacting with the protein species that are either functional
nuclei or their precursors. Hp does not bind to the native
monomer or to the mature fibrils formed from them, making
up a small family of extracellular chaperones that may be an
important part of an in vivo control system for extracellular
proteins [19].

The haptoglobin/hemopexin system normally binds extra-
cellular Hb and heme. However, the protective haptoglobin/
hemopexin/albumin/heme oxygenase systems can be over-
whelmed in disease states, including hemolysis and inflam-
mation resulting in increased circulating levels of free Hb and
free (or LDL bound) heme that can attack the endothelium
and cause perfusion. This and several reports indicate that
haptoglobin is increased in AD plasma and CSF. Recent evi-
dence indicates the Aβ-heme complex acts as a peroxidase,
resulting in increased oxidative damage.
Fetal (Hb F) expression, restricted to a subset of erythrocytes
(F cells), varies with genetic and environmental factors. Be-
cause methHb F does not bind Aβ1-42 as avidly as methHb A,
its interaction with Aβ results in less toxic, red blood cells
(RBC) lysis and damage of the vascular endothelium.
Decreased RBC lysis and Hb/heme mediated damage to the
microvasculature may help reduce hyperperfusion and
inflammation in the brain. Hb F may be less toxic than adult
Hb in its interaction with Aβ and may protect against the de-
velopment of AD [15].

Some other signaling molecules include neurosecretases,
family of metalloproteinases of the ADAMs family, “Alzheim-
er Associated Protein” (ALZAS), and “Neural Thread Protein”
(NTP) [8, 9, 11].

Markers typically used for non-AD dementive disorders in-
clude 14-3-3 proteins, neuron-specific enolase, S-100 pro-
tein, heart-type fatty acid-binding protein (H-FABP) [14].
The β-site APP cleaving enzyme-1 (BACE 1) is rate-limit-
ing for ultimate release of Aβ; it is a plausible target for
therapy especially since enzyme deletion is without effect
on neuronal development Concentration of this enzyme is
increased in the brain as result in AD, ageing, ischemia and
cerebral amyloid angiopathy.

The use of APP as a prototypic type-1 substrate provides
insights in enzymology of converting enzymes and their role
for regulated intramembrane proteolysis. This way was dis-
covered a new family of converting enzymes (BACE 1 and
2), existence of multimeric complexes γ-SC for final release
of Aβ, for export to form extracellular plaque.

The ADAMs (a disintegrin and metalloproteinase) are a family
of transmembrane and secreted proteins with important roles
in regulating cell phenotype via their effects on cell adhesion,
migration, proteolysis and signalling. ADAM acts as a putative
α-secretase to release nonamyloidogenic products.

The following members of the ADAM family have been impli-
cated in APP turnover (ADAM 9, ADAM 10, ADAM 17, ADAM
8). The domains structure of TACE (ADAM 17) provides
a basis for identifying sites for drug development. ADAM 17 is required for generation of the active forms of Epidermal Growth Factor Receptor ligands, and its function is essential for the development of epithelial tissues [8].

Alzheimer Associated Protein (ALZAS) a novel Aβ protein expressed in elderly patients with the diagnosis of probable AD was discovered on chromosome 21 within the APP region. ALZAS with a 79 amino acid sequence contains the Aβ-42 fragment, the AP transmembrane signal, and a unique 12 amino acid C-terminal. ALZAS has its starting codon within the exon 16 and its coding sequence ends in intron 17. The expression of the transcript of this protein was found in cortical and hippocampal brain regions as well as in lymphocytes of AD patients.

Regulating the access of α and β-secretases to APP and ALZAS seems to be of importance that Aβ-fragments (truncated Aβ1-16, 1-33, 1-39, 1-42) in CSF distinguish sporadic AD from non-demented controls with accuracy of 86%. ALZAS with its β-helical structure may be a substrate for α-secretase, could act as molecular chaperone that binds APP and assists in altering its conformation. In serum of patients with clinically probable AD up to tenfold increase of the ALZAS antibody titer directed against the C-terminal, non-amyloid terminal was found revealing the highest titers in the early stages in patients with presymptomatic AD or MCI but moderate titers in fully developed AD. Maximal values were found in the sera of patients over age 65, who had been diagnosed as „depressed” without recognizable cognition disorders. Depression is common in early stages of AD (up to 87%).

The serum of AD patients contains increased concentration of ALZAS antibodies. Significant elevation of serum ALZAS IgG was found in patients with early stages of dementia. There is a considerable variability of the specificity and sensitivity of ALZAS protein. In the future, assessment of autoantibodies in the serum, blood platelets and lymphocytes against Aβ and receptor for advanced glycation-end products may appear of increasing interest and may indicate close relations between AD and autoimmune disorders. ALZAS protein in the serum in both, early and late stages of AD, is suggested to represent an indicator of a dynamic equilibrium between both peripheral and brain degenerative changes, thus providing a reliable and simple diagnostic marker for AD by a simple non-invasive blood test [11].

Neural Thread Protein (NTP) is a brain protein associated with the pathological changes in AD, brings about early neuronal cell death. Elevation of NTP has been found in AD brain, CSF and urine.

The utility of the measurement of NTP in the urine of patients presenting with cognitive symptoms was found. Diagnostic evaluation involves patients’ history, physical and and neurological examination, blood tests, neuropsychological assessment, structural brain imaging, and a number of possible procedures such as further blood, urine and CSF tests, genetic testing, EEG, functional imaging angiography; and rarely brain biopsy. There is a well-recognized need for simple testing methods in the initial assessment of patients with cognitive difficulties and possible dementia.

NTP fulfills most of the criteria for an „ideal biomarker”. One clinical study shows the utility of its measurement as a clinical tool for assessing likelihood of AD. With a cutoff of 22 µg/ml elevated urine NTP had a 91.4 % agreement with the clinical diagnosis of probable AD, 37.7% with possible AD, and 48.7% with MCI. Practical conclusions: the utility of these marker measurements in the workup of cases of dementia and AD was found [9]. For initial evaluation and for the nonspecialist practitioner it may be helpful in part as an early indicator, however with several limitations. These data clearly suggest that urine NTP adds value to the diagnostic process by increasing positive predictive value and negative predictive value. This noninvasive test is potentially helpful as part of the workup of dementia for the nonspecialist. The optimal clinical context for using urine NTP has yet to be clarified. Nymox Corporation sponsored this study, including performing the blinded UNTP measurements in its central core laboratory at no cost and reimbursing clinical sites for the time and additional imaging studies [9].

A simple urine test to aid the diagnosis of AD could revolutionise the way patients with the disorder are diagnosed and treated. The company thinks that it has developed a urine test that works, but some in the academic community remain unconvinced [3].

Markers typically used for non-AD dementia disorders [14] include:

• 14-3-3 proteins.

These proteins belong to a protein family with a molecular weight of around 30 kDa, with at least seven isoforms which show a highly conserved amino acid sequence in almost all Eucaryota species. They are attributed to play a role in signal transduction, especially in the mediation of binding between kinases. Patients with possible 14-3-3 in CSF fulfill clinical criteria for probable Creutzfeldt - Jakob disease.

• Neuron-specific enolase (NSE)

In the CSF, 98% of the enzyme originates from the central nervous system. As one of the first surrogate markers a cutoff value for NSE in CSF is 15 ng/ml at which patients can be diagnosed with 78% sensitivity and 88% specificity as CJD cases. The differential diagnostic use for dementia is low [10].

• S-100 protein

The determination of S-100 protein has recently assumed increasing diagnostic importance as a tumor marker in malignant melanoma, in evaluating the prognosis of ischemic cerebral infarction, and in assessing the development of neuropsychological deficits after minimal head trauma. Elevated S-100B values were found in CSF from CJD patients and were significantly higher than in patients with dementias of other origin. Serum S-100B level of AD patients might be more suitable as a marker of progression than as a diagnostic marker.
Inflammatory, immunology systems

The increased lipid peroxidation products suggest that oxidative stress has an important role to play in the pathogenesis of neurodegenerative diseases. Reactive oxygen species react with polyunsaturated fatty acids to induce the release of toxic and reactive metabolites. In the central nervous system extracellular matrix metalloproteinases can be released from astrocytes, neurons, oligodendrocytes, microglia, endothelial cells and and leucocytes. Inflammatory mediators may act as inhibitors of matrix metalloproteinases as demonstrated by the ability of IFN-gamma, IL-4 and IL-10 to suppress their synthesis. This suggests that they are both effectors and regulators of the inflammatory response. Pro-and anti-inflammatory cytokines play an important role in AD, and common polymorphisms of genes controlling their production have been shown to be associated with the susceptibility to sporadic AD [20].

Changes in the functions of of immune system are common in AD and healthy elderly subjects (also a decline in T cell number and function and in B cell number). An increase in CD8+CD28 - suppressor cells and decrease in cells expressing CD69 and CD71 was reported. In AD elevated levels of IL6 in plasma, increased production of IFN-γ and TNF-α by NK cells, and an increase in IL-1β associated with a decrease in IL-10 and CD8+ from mild to moderately severe AD were observed. In comparison with the HC (healthy cases) the absolute number and percentage of CD6+CD71+ cells were significantly higher among the AD patients and particularly in the moderately severe AD. Compared to HC the AD group showed a significant decrease of CD8+CD28. This increase may suggest activation in MHC class-I-restricted CD8 cells and indicate the potential involvement of iron homeostasis in AD. A reduction of CD8+CD28-T cells in AD compared to healthy may represent a decrease in suppressor activity that is particularly evident in the number of circulating cells in mild and moderately severe AD.

The presence of an exacerbated decrease of B cells in AD as in ageing but at the same time was found an immune activation through increasing CD8-activated cells expressing CD71 and CD28 and decreasing levels of CD8+CD28-suppressor was found in IL-10 production. Significant decrease in IL-10 production in all the AD groups after stimulation with only β1A-40. This may be important as therapeutic targets to recover or ameliorate the anti-inflammatory pathway that seems to be altered in AD [17].

The presence of inflammatory cytokines in the brain of AD patients does not seem to be merely a consequence of the degenerative process, but it appears to play a role in the cascade of events inducing neuronal death. Plasma levels of cytokines follow the degree of AD suggesting a gradual decline of immune responsiveness in AD [13].

IL-16, a growth factor for resting CD4+ cells, stimulates the production of inflammatory cytokines IL-1β, IL-6 and TNF-α, induces a rise of intracellular Ca++ or inositol-(1,4,5)-triphosphatase and translocation of the protein kinase (PKC). The elevation of IL-16 levels in the early phases of AD contributes to chemoattraction of pathogenic CD4 lymphocytes across the blood-brain barrier (BBB). Therefore IL-16 could play a crucial role in the regulation of signaling between the brain and the immune system.

IL-18 is a potent proinflammatory member of the IL-1 superfamily, and increasing evidence indicates a crucial role for it in the pathogenesis of AD. Binding of IL-18 to its receptor expressed on resident and infiltrating cells in CNS leads to activation of the transcription factor NF-kB via a complex intracellular signaling cascade. IL-18 may be one of the apoptosis-inducing factors mediated by TNF-α leading to the neurodegeneration during the AD progression. Two functional polymorphisms in IL-18 promoter (-607C and -137G alleles) are associated with an increased risk of developing sporadic AD. These associations showed a highly significant synergistic interaction with ApoE ε4 allele [20].

IL-18 induces production of T helper 1 (Th1) cytokines. Stimulation with IL-18 and IL-12 of Th1, NK cells, activated B cells and macrophages/microglia makes them potent IFN-γ producers. TGF-β1 is a potent immunosuppressive cytokine and has a pivotal role in the control of the transition between proinflammatory (Th1-type) and anti-inflammatory (Th2-type) response. Overexpression of TGF-β1 may initiate or promote amyloidogenesis in AD and be a risk factor for developing AD. An increased production of APP and subsequent Aβ production in astrocyte cultures in response to TGFβ-1 was reported [20].

TNF-α, a proinflammatory cytokine, and the pleiotropic hormone insulin-like growth factor (IGF-I) have been involved in the pathogenesis of AD as neurotoxic and survival factors, respectively. Elevated levels of TNF-α and related molecules were observed in the brain and CSF of AD patients, whereas IGF-I gene expression was found to be markedly reduced in AD brains [2].

TNF-α levels correlated negatively with total and free IGF-I values in AD patients and to a lesser extent in MCI subjects. The increase of TNF-α might be involved in the pathogenesis of AD and MCI. TNF-α might antagonize the potential neurotrophic activity of IGF-I in AD. The combined determinations of TNF-α and IGF-I might be useful to monitor anti-inflammatory and/or neurotrophic drug effects in AD [2].

Conclusions

Although AD has been studied for one hundred years, the effort to establish diagnostic criteria and drug discovery toward delay the onset, disease prevention or modification begun
approximately less than twenty years ago. The early and accurate detection of dementia symptoms in the prodromal neurodegenerative processes have been the subject of multiple scientific research.

Rapid progress toward understanding the molecular bases of neurodegenerative disorders such as AD, PD and other dementias is changing drug discovery for these conditions and disease-modifying therapies. However, there is an urgent need for biomarkers to diagnose these disorders early in their course, when therapy is likely to be the most effective, and to monitor responses of patients to new therapies. The clinical diagnosis and design of therapy are especially difficult in the patients with MCI when some symptoms of normal aging may constitute starting point as predictors of prodromal phase of AD and other dementias.

Biomarkers for neurodegenerative disorders can be divided into markers of disease state and markers of disease rate. The combination of regional cerebral blood flow (rCBF) (parietal cortex) and CSF biomarkers clearly improves the risk assessment of future AD in subjects with MCI [10].

Inflammatory mechanisms may play an important role in neurodegeneration of AD as various inflammatory mediators have been reported in AD brains, and epidemiological studies have been reported altered AD risk with the use of anti-inflammatory drugs. The field of AD biomarkers has experienced a renewed level of enthusiasm due to better availability of reagents and novel techniques to assess a variety of body fluid markers and imaging measures. For example CSF Aβ1-42 and tau remained the timeless tests, proving particularly promising as potential predictors of cognitive decline in individuals with MCI as well as future dementia in non-demented persons. Such a combination is already sufficient in the diagnosis of AD, but these markers are not specific for a single form of dementia. Further research in this field is necessary.

Besides plasma, serum, urine, CSF used as biological material the development of „molecular” imaging agents for the detection of AD pathology (amyloid, tangles, activated microglia) has propelled the imaging field forward, especially combinations of fluid and imaging measures will be used for the design and evaluation of clinical trials of disease-modifying therapies by helping to reduce sample size, reduce trial duration, and evaluate treatment efficacy.

The use of nanobiotechnology, combination of amyloid-β-specific monoclonal antibodies with an ultrasensitive, nanoparticle-based protein detection strategy termed bio-barcode amplification enables simultaneous detection of several targets at attomolar (10^-18) concentration. Therefore identification of particular biomarkers will be achieved by unbiased strategies based on proteomics, metabolomics, pharmacogenomics or related technologies.

References

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