Alzheimer's disease, inflammation and non-steroidal anti-inflammatory drugs

Ferencik M, Novak M, Rovensky J, Rybar I

Abstract

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Alzheimer's disease (AD) is a degenerative disease of the brain, which causes dementia. The disease is characterised by three main pathogenic factors: senile plaques, neurofibrillary tangles and inflammation. The participation of the local inflammatory reaction is confirmed especially by the results of studies dealing with activated microglia, reactive astrocytes, complement system, cytokines, reactive mediators of oxygen and nitrogen (free radicals), all of which participate significantly in inflammatory processes. These inflammatory markers are locally produced by brain cells, and occur in close proximity of beta-amyloid and tau protein deposits. Moreover, some epidemiologic and pilot clinical studies have proven that long-term administration of anti-inflammatory drugs have a protective effect on the onset of AD. Out of them, non-steroidal anti-inflammatory drugs (NSAIDs) are most extensively investigated medicaments. Despite some contradictory findings, the prevalent majority of these studies prove that long-term application of anti-inflammatory treatment can delay the onset, or at least slow down the progression of AD, namely in people between 65 and 75 years of age. The most appropriate prophylactic effect seems to be achieved by specific inhibitors of cyclooxygenase-2 (COX-2), namely celecoxib and rofecoxib. These preparations protect the gastrointestinal tract better than classical NSAIDs which inhibit both isoenzymes — COX-1 and COX-2. COX-2 is expressed in higher concentrations in the degenerating cells of the brain and this excessive expression can be decreased by selective inhibitors. The latter decrease also the excessive activation of some transcription factors (PPARgama and the nuclear factor kapa-B), which are responsible for the initiation of transcription of a number of pro-inflammatory genes. The selective inhibitors

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Abstrakt

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Alzheimerova choroba (ACH) je degeneratívne ochorenie mozgu, ktoré spôsobuje demenciu. Chorobu charakterizujú tri hlavné patogenetické faktory: senilné plaky, neurofibrilárne klbká a zápal. Účasť lokálnej zápalovej reakcie dokazujú najmä výsledky štúdií s aktivovanou mikrogliou, reaktívnymi astrocytmi, komplementovým systémom, cytokínmi, reaktívnymi intermediátmi kyslíka a dusíka (voľné radikály), ktoré všetky sú významnými účastníkmi na zápalových procesoch. Tieto zápalové markery lokálne produkujú mozgové bunky a nachádzajú sa v tesnej blízkosti depozitov beta-amyloidu a tau-proteínu. Okrem toho niekoľko epidemiologických a pilotných klinických štúdií dokazuje, že dlhodobé podávanie protizápalových liečiv má protektívny účinok na vznik ACH. Z týchto preparátov sa najčastejšie sleduje pôsobenie nesteroidných protizápalových liečiv (NSAIDs). Napriek niektorým protichodným nálezom prevažná väčšina týchto štúdií dokazuje, že dlhodobá aplikácia protizápalovej liečby môže oddialiť začiatok alebo aspoň spomaliť progresiu ACH, a to osobitne u osôb medzi 65. a 75. rokom života. Na takéto profylaktické použitie sa zdajú najvhodnejšími špecifické inhibítory cyklooxygenázy 2 (COX-2), ako je celekoxib a rofekoxib. Tieto preparáty majú oveľa lepšiu gastrointestinálnu bezpečnosť ako klasické NSAIDs, ktoré inhibujú oba izoenzýmy — COX-1 aj COX-2. COX-2 sa vo zvýšených koncentráciách exprimuje v degenerujúcich oblastiach mozgu a túto nadmernú expresiu môžu znížiť selektívne inhibítory COX-2 rovnako ako nadmernú aktiváciu niektorých transkripčných faktorov (PPARgama a nukleárny faktor kapa-B), ktoré zodpovedajú za iniciáciu transkripcie mnohých prozápalových génov. Selektívne inhibítory COX-2 môžu tak pôsobiť protizápalovo na viacerých úrovniach. *(Tab. 1, obr. 1, lit. 75.)*

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COX-2 can thereby have an anti-inflammatory effect operating on several levels. ***(Tab. 1, Fig. 1, Ref. 75.)***
***Key* words: Alzheimer's disease, inflammation, complement, cytokines, acute phase proteins, free radicals, microglia, astrocytes, cyclooxygenases, non-steroidal anti-inflammatory drugs.**

Kľúčové slová: Alzheimerova choroba, zápal, komplement, cytokíny, proteíny akútnej fázy, voľné radikály, mikroglia, astrocyty, cyklooxygenázy, nesteroidné protizápalové liečivá.

One of the most essential questions which were discussed at the World Congress on Alzheimer's Disease (AD) in 2000 in Washington, was the role of inflammation in coincidence with this disease. The recent 15 years have been rich in the amount of immunologic histochemical, biochemical and molecular immunologic data proving that AD brain contains typical inflammatory mediators, while in the brain of the aged not showing signs of dementia, these molecules are practically not detectable. Experimental and clinical studies have confirmed that, the inflammatory process significantly participates in neurodegenerative changes associated with AD. Due to this fact, an essential question has arisen: If the damage of neurones and subsequent dementia in AD are brought about due to inflammatory mechanisms, can anti-inflammatory drugs be possibly successful in AD prevention and/or therapy?

In order to re-evaluate the number of data dealing with inflammatory reactions participating in the pathogenesis of AD (the data published sooner than 10 years ago are often contradictory), and to answer the question as to the possible application of antiphlogistic drugs in this disease, the Neuroinflammation Working Group consisting of 37 world experts was established. The results of their work were presented at the above mentioned congress in form of an open study (Akiyama et al., 2000).

The subjective of this study is to review the current knowledge on the role of inflammation in AD, namely from the aspect of the results presented at the congress in Washington, and deliberating the facts presented in medical literature prior to 2000.

Factors initiating the inflammatory process within the brain of patients with AD

Despite some of its specific properties, the brain, is not an immunologically isolated organ, and therefore it can develop a defensive, as well as damaging inflammatory reaction (Blalock, 1994; Ferenčík et al., 1998). Although many questions have remained unclear, it has been considerably proven that while lymphocytes and monocytes are typical inflammatory cells in the periphery, they participate to no or to little extent in brain inflammation which is usually of local character. Conventional non-steroidal anti-inflammatory drugs (NSAIDs) also referred to as non-steroidal antiphlogistic drugs (NSA) can either delay or slow down the progression of AD (McGeer et al., 1996).

The majority of research studies dealing with the role of inflammation in AD were performed by groups of expert immunologists, specialised namely in cytokines including chemokines, the complement, growth factors, oxidation stress, microglia, microglial activation, etc. The effects of inflammatory mediators and cells within the human organism are not brought about separately, but as a complex which mutually reacts in many ways. Therefore, in order to comprehend the reasons of the inflammatory response correctly, it is necessary not only to know the changes on the level of individual mediators, and functions of appropriate cells, but also their timing and mutual positive and negative interactions.

The signs of damage or gradual disappearance of cognitive and intellectual functions taking place in AD, are associated with three main pathogenic factors: formation of senile neural plaques, neurofibrillary tangles, and the inflammatory process. The main component of senile plaques is β-amyloid peptide (Aβ) formed by 39—43 amino acid units (Dickson, 1997). It is produced by enzymatic splitting of β-amyloid precursor protein (βAPP). Under normal conditions, Aβ is soluble, but in AD brain, in result of conformation changes, it forms insoluble fibres which deposited in extracellular space, thus forming so-called senile plaques. Neurofibrillary tangles contain excessively phosphorylated short forms of tau protein which in this form also becomes insoluble and resistant to degradation. It stops fulfilling its microtubular stabilisation function, and aggregates in neurotoxic tangle structures filling the interior of neurones (Novák, 1997).

Aβ and tau are two key molecules, the pathologic forms of which trigger the inflammatory processes, or other pathogenic mechanisms resulting in AD. The group of authors who, in this problem, prefer Aβ are referred to as "βaptists" while the other group is referred to as "tauists". However, the reasons which trigger the pathological changes in these two molecules are not known to full extent. For instance, in case of tau protein, they are triggered by extensive phosphorylation and the existence of isoforms (Novák, 1997). The question, as to what activates the specific kinases — enzymes which catalyse such phosphorylation, is in place. The origin of inflammation and subsequent development of AD is affected by various risk factors, especially by age and genotype containing genes or their alleles which increase the sensitivity of individuals to the origin of pathologic forms of Aβ and tau, as well as to the inflammatory process, either in general or separately in the brain.

Inflammatory mediators in the brain of patients with AD

Assumedly, Aβ and tau mutually participate in the onset of inflammatory process in AD brain. This mutual participation is evident from several facts. For instance, Aβ as well as tau proteins can activate the *complement system (C)* in their classical way, namely directly without the presence of antibodies and thereby in the absence of the appropriate immune complexes. The activated C1 component then markedly increases the aggregation of Aβ and tau, thus enclosing the vicious circle: the more Aβ aggregates, the more C activates. The more C activates, the more Aβ aggregates. Besides, Aβ in conformation of β pleated sheet (β structure) activates the complement in an alternative pathway. The C3 and C5 fragments formed by activation, represent chemotactic and activation factors for inflammatory cells which have specific receptors for these fragments. Such receptors are also on the surface of microglia and astrocytes. The complement comes from local sources, since neurones, microglia and astrocytes can synthesise all its components and factors. Their secretion is increased during inflamma-

tion. The membrane attack complex – $C5b678(9)_n$ originating by C activation can then affect the surrounding neurones, or other cells cytotoxically or even cytolytically (McGeer and McGeer, 1999; Gasque et al., 2000). The production of components and factors of C in AD brain can be as extensive as in the liver which is the primary peripheral source of C (Yasojima et al. 1999). The participation of C in activation of inflammation is proven by the fact that in AD brain, a number of activated C fragments can be proven in the surroundings of plaques with aggregated Aβ, or a number of neurofibrillary tangles with aggregated tau, as well as reactive astrocytes and activated microglia (Akiyama et al., 2000).

Cytokines

The brain of AD patients contains increased levels of practically all inflammatory cytokines including IL-1, IL-6, IL-8, TNF-α, MIP-1 which activate especially the microglia and astrocytes. The increased concentration of IL-1 influences the formation of plaques and regulates their principal development especially by means of stimulating the βAPP synthesis (Mackenzie, 2000). IL-6 is synthesised by microglia, astrocytes, neurones as well as endothelial cells within vascular microcirculation. Aside from the inflammatory effect (which is manifested especially by inducing the production of acute phase proteins, by affecting in the function of endogenous pyrogen, increasing the vascular permeability and activation of lymphocytes), IL-6 can, in certain situations, have an inflammatory and immunosuppressive effects. It stimulates e.g. the hypothalamus-pituitary-adrenal axis, and thus, also the production of glucocorticoids which then have a systemic immunosuppressive effect (Mastorakos, 1993). The IL-6-encoding gene of each individual contains two of several possible alleles, and can encode at least 6 variously active isotypes of this cytokine. Some alleles encode isotypes which more willingly participate in inflammatory reaction (Akiyama et al., 2000). Individuals who own such an allele are more likely to develop an inflammatory reaction than those who do not posses it. This illustrates the complexity of the effect of cytokines, should their activity be judged from one singular aspect.

The level of typical alarm inflammatory cytokine — tumour necrosis factor – α (TNF-α) is increased not only in the brain of patients with AD, but also in their spinal cord and blood serum. Its pathophysiologic effects are, however, controversial. While in the periphery TNF-α acts as an inflammatory and cytotoxic mediator, within CNS, it can have neuroprotective effects. Their basic function can reside in the ability of TNF-α to induce the production of cytoprotective molecules within the neurones, as e.g. superoxide dismutase containing the manganese which splits down superoxide, and thereby protects the neurone from oxidation damage (Bruce-Keller, 1999).

The transforming growth factor β (TGF-β) produces three active isoforms, the receptors of which are found on neurones, astrocytes and microglia cells. TGF-β can influence AD pathogenic processes, including the inflammatory response, microglial activation, astrocytosis, neurone damage, formation of extracellular matrix, accumulation and local division of Aβ, regulation of known or potential risk factors of AD (βAPP, ApoE, cyclooxygenase 2) and apoptosis inhibition. In AD, TGF-β occurs in plaques and its increased levels are in brain medullar fluid and in blood serum. Despite the fact that TGF-β is known as an anti-inflammatory cytokine, in the brain it can have, under certain pathological conditions, inflammatory effects possibly based on the function of the chemotactic factor for microglia cells (Yao, 1990), stimulation of PGE_2 synthesis and the expression of cyclooxygenase 1 (COX-1) and COX-2 in neurones, as well as in astrocytes (Luo et al., 1998).

It is not easy to define the role of individual cytokines in the development of inflammation within the brain of patients with AD. The effect of none of cytokines is isolated. Each cytokine works in complex with further cytokines, out of which some can have a potentiating effect, the others can function as inhibitors. Such a group of cytokines forms a cytokine network. Its resulting effect is dependent on many variables (the presence of particular cytokines, their receptors, antagonists and inhibitors in the given anatomical site and time, the velocity of their secretion and degradation, genetic profile formed by possible isotypes) which can differ not only in individual organs and tissues, but also in time and clinical situations.

However, from the pathogenic point of view, the following facts about the brain of AD patients are important:

a) there are increased concentrations of many inflammatory cytokines including chemokines (Steng et al., 1996; Xia et Hyman, 1999; Lue et al., 2000);
b) interactions of some cytokines with Aβ, or tau proteins can have pathophysiological significance (Suo et al., 1998; Meda et al., 1999);
c) CNS cells are able to synthesise and secrete many cytokines (Tab. 1).

Tab. 1. Cytokines produced by cells of the CNS.
Tab. 1. Cytokíny, ktoré môžu produkovať bunky CNS.

Cells Bunky	Cytokines Cytokíny
Microglia Mikroglia	IL-1, IL-6, TNF-α, TGF-β1, TGF-β2, TGF-β3, MIP-1α, MCP-1
Astrocytes Astrocyty	IL-1, IL-6, IL-8, TGF-β1, TGF-β2, TGF-β3, GM-CSF, M-CSF, G-CSF, MIP-1α, IFN-γ
Oligodendrocytes Oligodendrocyty	TGF-β
Neurons Neuróny	IL-1, IL-6, TGF-β1, TGF-β3
Endothelial cells in brain vessels Endotelové bunky mozgových ciev	IL-1, IL-6, IL-8, IL-11, IFN-α, IFN-β, IFN-γ, TGF-β, MIP-1

IL — interleukín, TNF — faktor nekrotizujúci nádory (*tumor necrosis factor*), TGF — transformujúci rastový faktor (*transforming growth factor*), MIP — makrofágový zápalový proteín (*macrophage inflammatory protein*), MCP — monocytový chemotaktický proteín (*monocyte chemoattractant protein*) GM-CSF — faktor stimulujúci kolónie granulocytov a makrofágov (*granulocyte-macrophage colony stimulating factor*), M-CSF — faktor stimulujúci kolónie makrofágov (*macrophage colony stimulating factor*), G-CSF — faktor stimulujúci kolónie granulocytov (*granulocyte colony stimulating factor*), IFN — interferón.

Leukoadhesive molecules

Despite the fact that inflammation of AD brain does not display leukocyte infiltration which is typical of peripheral tissues, the leukoadhesive molecules responsible for transendothelial migration of leukocytes, as β_2-integrin molecules, can possibly participate in the inflammatory process also in AD brain.. This is assumed due to the fact that LFA-1, CR3 and CR4, typical β_2-integrin molecules occur to a greater extent on the surface of activated microglia cells in the surrounding of β-amyloid plaques and astrocytes which are in their proximity, express ICAM-1 (intercellular adhesive molecule, member of the immunoglobulin superfamily) which is the ligand for LFA-1 (Akiyama et al., 2000).

Reactive forms of oxygen

Free radicals derived from oxygen are considered to represent the inflammatory and injuring molecules which participate also in pathological processes in AD (Behl, 1999; Markesbery and Carney, 1999). The key enzyme which produces superoxide within peripheral neutrophils and macrophages, is NADPH oxidase. From superoxide further reactive oxygen intermediates (ROI) can be originated (Bergendi et al., 1999; Ferenčík et al., 2000). NADPH oxidase is a complex enzyme which, in AD, is activated in microglia (Shimohama et al., 2000) and ROI (particularly hydrogen peroxide) which subsequently originate, damage the surrounding neurones. The essence of this toxic effect and subsequent loss of neurones is based on mutations of mitochondrial DNA which result in an irreversible loss of mitochondria (Delamonte et al., 2000). Thereby, the neurones lose their energetic centre. The activation of NADPH oxidase in the brain of patients with AD is the responsibility of Aβ polypeptides (McDonald et al., 1997; Van Muiswinkel et al., 1999).

The pathogenesis of oxidation stress which takes place by means of ROI has an impact on various neurodegenerative processes including AD. This process is of long-term character, and ROI, in AD, are produced especially in microglia activated by Aβ. However, it should be emphasised that Aβ is continuously produced in the brains of both healthy people and AD patients. Under normal conditions, however, Aβ within the brain is replaced sooner than it could produce insoluble extracellular fibres representing the agents able to activate microglia and induce the production of ROI. This process takes place by the already mentioned activation of NADPH oxidase by means of two types of receptors. One of them is the receptor for advanced glycation end-products (RAGE), which is situated on the surface of microglia cells, neurones and endothelial cells (Yan et al., 1996). The other is a scavenger receptor — SR (El Khoury et al., 1998) which mediates endocytosis and degradation of modified LDL and glycated ligands including the tau protein. The expression of both receptors is markedly increased in afflicted areas of AD brain (Akiyama et al., 2000).

Reactive forms of nitrogen

In addition to ROI, the pathogenesis of AD is affected by nitric oxide and other reactive nitrogen intermediates (RNI). Nitric oxide in its radical form ($NO^{\bullet}$) is produced by NO synthases (NOS) — enzymes which occur in three main isoforms, as neural (nNOS), endothelial (eNOS) and inducible (iNOS) NO synthases. High, and thus cytotoxic concentrations of $NO^{\bullet}$ are produced especially by the activity of iNOS (Bergendi et al., 1999; Ferenčík et al., 2001). Several cytokines (e.g. IL-1, IFN-gamma) induce expression of iNOS in astrocytes which then produce NO in concentrations damaging the surrounding neurones (Chao et al., 2000). The astrocytes of patients with AD display also an increased expression of nNOS, and this increase correlates with the injury of DNA and the number of dead neurones within the hippocampus and entorinal cortex (Simic et al., 2000). The data on induction of iNOS expression in microglia of patients with AD are controversial. However, the recent experiment (Ishii et al., 2000) proves that Aβ polypeptide directly stimulates the production of NO by glial cells in AD brain. Selective inhibitors of iNOS decrease the production of NO induced by Aβ, and therefore, they possibly could take part in the prevention and therapy of AD.

Microglia represent the site of superoxide production, which reacts with $NO^{\bullet}$ resulting in the production of very cytotoxic peroxynitrites which can give rise to even more toxic hydroxyl radical $^{\bullet}OH$. The marker of the effect of peroxynitrites is nitration (introduction of the $-NO_2$ group into the molecule) of tyrosine units in the molecules of proteins. Nitrotyrosine units are found in the brain neurones of patients with AD, however not in the neurones of normal brains. In normal brain, NO can be produced also in neurones and endothelial cells.

Microglia in AD brain expresses significant amounts of myeloperoxidase (Reynolds et al., 1999), which together with hydrogen peroxide and chloride anions form the myeloperoxidase system, the activity of which produces toxic hypochlorite and active chlorine (active components of chloride of lime).

In addition to its direct injuring effect on neurones in AD brain, $NO^{\bullet}$ is also an activating factor of the programmed cell death — apoptosis and interferes the production of prostaglandins by cyclooxygenase isoenzymes (Clancy et al., 2000).

Acute phase proteins

Since the production of acute phase proteins (APP) is activated by a number of cytokines and other inflammatory mediators, it is not surprising, that many of APP are found within the proximity of senile plaques and extracellular neurofibrillary tangles. They include serum amyloid protein — SAP, C reactive protein — CRP, α_1-antichymotrypsin (αACT) and α_2-macroglobulin. αACT occurs not only within the deposits of Aβ peptide in AD brain, but stimulates also the transformation of its non-fibrillary form into a fibrillary insoluble form (Ma et al., 1999). Similar situation is applied with α_2-macroglobulin functioning as a wide-range inhibitor of proteolytic enzymes. It do not only occur in the proximity of plaques and neurofibrillary tangles, but also form complexes with Aβ which can be engulfed by phagocytes (Navita et al., 1997) thereby inhibiting Aβ aggregation and formation of insoluble fibres. This ability is owned also by other APP including C1q, apolipoprotein E and αACT.

The fact that apolipoprotein E (ApoE) is a significant risk factor in AD development, has been known for a long time. Its gene is located on chromosome 19. Out of all its individual isoforms, the liability to AD development is linked especially with ApoE4 (Strittmatter et al., 1993), namely in homozygotes (ApoE4/4). The mechanism, by which ApoE4 affects this process is still not well understood. It is assumed, however, that the ApoE4 isoform is not

able to react with tau protein, in result of which the latter can undergo partial degradation, and, consequently, its polypeptide chain can be shortened. The shortened tau forms aggregate spontaneously, thus forming Alzheimer's pair spiral fibres which are the main components of neurofibrillary tangles. Other isoforms, especially ApoE3, have the function of chaperons. They bind with tau protein and thus protect it from pathologic degradation (Novák, 1997).

Prostaglandins, thromboxanes and cyclooxygenases

Prostaglandins (PGs) and thromboxanes (TXs) are significant inflammatory mediators, which are synthesised within the arachidonic acid cascade in effect of cyclooxygenase (COX). This enzyme occurs in two isoforms — COX-1 and COX-2 (Fig. 1), the genes of which are located on various chromosomes (O'Banion et al., 1992; Lipsky, 1999). COX-1 is a constitutive enzyme produced by many types of cells, and its products take part especially in the regulation of normal physiologic processes. COX-2 is also a constitutive enzyme, but its expression within inflammatory cells can be increased 20-fold by induction, especially of several inflammatory mediators (IL-1, TNF-α, ROI, RNI), therefore it can be considered as an inductive enzyme (Mitchel et Warner, 1999). Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the activity of both COX-1 and COX-2. The inhibition of COX-1, however, can consequently inflict damage on alimentary mucosa and disturb the functions of the kidneys and thrombocytes. Therefore, NSAIDs are considered as "ideal" in case that they can inhibit solely the inflammatory COX-2 (Vane et al., 1998; Hawkey, 1999).

It has been found out that the brain of patient with AD contains increased levels of COX-2, and the latter are directly proportional to Aβ levels, density of amyloid plaques, and to atrophy of neurones (Ho et al., 1999). COX-2 occurs especially in neurones, where its increased activity can lead to their direct death via free radicals, or to apoptosis via stimulation of apoptotic factors. The inhibition of COX-2 by means of antiphlogistic drugs can be therefore beneficial in patients with AD.

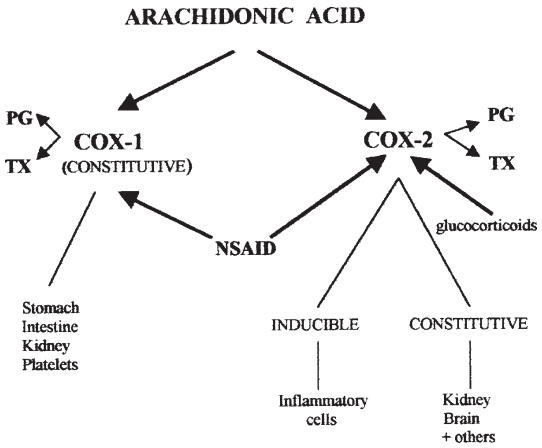

Fig. 1. Production of prostaglandins (PG) and thromboxanes (TX) by cyclooxygenase isoenzymes (COX-1 and COX-2) in different cells and organs.
Obr. 1. Tvorba prostaglandínov (PG) a tromboxánov (TX) izoenzýmami cyklooxygenázy (COX-1 a COX-2) v rôznych bunkách a orgánoch.

The cells producing inflammatory mediators in the brain of patients with AD

It is assumed that the key role in the initiation of inflammatory processes within the AD brain is played by microglia activated by insoluble aggregates of Aβ and/or of tau protein (Akiyama et al., 2000). This assumption is confirmed by the facts that activated microglia cells secrete an amount of inflammatory cytokines (Tab. 1) and other mediators, and that the expression of HLA antigens class II on their surface is increased — similar to that on the surface of activated peripheral macrophages (Styren er al., 1990; Streit et al., 1999). Astrocytes within AD brain are not expressed by class II HLA antigens, and therefore, to refer to them as being "reactive" is more precise, than to use the term "activated" (Akiyama et al., 2000).

In addition to inflammatory cytokines, activated microglia cells secrete also the components of complement, oxygen and nitrogen intermediates (ROI and RNI), proteolytic enzymes, excitatory amino acids and other mediators which not only activate the course of the damaging inflammatory reaction, but a number of them can have a direct neurotoxic effect. The fact that the microglia in the neighbourhood of senile plaques has increased levels of phosphotyrosine suggests that microglia has permanently increased intracellular transmission signal cascades using tyrosine kinases. Protein kinases operating in these cascades phosporylate the transcription factors which subsequently activate the transcription of inflammatory genes. Protein kinase signal cascades also activate the NADPH oxidase within the microglia. This oxidase catalyses the production of superoxide which gives rise to other reactive forms of oxygen conditioning the local oxidation stress. These mechanisms are used by Aβ and/or tau proteins in AD brain not only in order to trigger the damaging inflammation, but also to maintain its chronicity. The mentioned facts imply that all drugs, either inhibiting or antagonising this complex inflammatory process, could possibly have preventive or therapeutic effects in AD.

Microglia assumedly participates also in the origin and development of neuritic plaques, namely by means of synthesis, processing or catabolism of β-amyloid precursor protein (βAPP) or Aβ peptide, as well as of tau protein, the pathologic form of which, in neuritic plaques, correlates significantly with the stage of cognitive damage in AD (Novák et al., 1999). The production of βAPP has not been confirmed in microglia of AD brain. The primary source of Aβ within the brain are the neurones (Akiama et al., 2000). Microglia can however turn the soluble form of Aβ coming from other sources into insoluble fibres which form a part of pathologic neuritic plaques. In this way, their function would be similar to that of peripheral macrophages in systemic amyloidosis. This is proven by the fact, that microglia is found around the neuritic plaques to a much greater extent than around the diffuse plaques in the brain of the aged who do not suffer from dementia. The diffuse plaques, similarly, contain neither the pathologic forms of tau protein. This fact represents further evidence of their significant role in the pathogenesis of AD (Novák et al., 1999).

It is probable that the formation of plaques in AD brain is a dynamical process where microglia can participate in the formation of insoluble fibres of Aβ, but also in their replacement by the mechanism of phagocytosis. The fibres of Aβ within the plaques are opso-

nised by iC3b enabling their bond with complement receptors (CR1 and CR3) on the surface of microglia cells, and subsequent phagocytosis (Bradt et al., 1998). Phagocytosis of aggregated forms of Aβ, however, does not have to be only a beneficial process as the phagocytes undergo the respiratory burst which produces cytotoxic free radicals. In addition to their direct cytotoxic effect, some of them can also bring about apoptosis (programmed death) of the surrounding cells. Recently, it has been found out (Fasulo et al., 2000) that the pathologic forms of tau proteins not only represent the substrates in the programmed cellular death, but can act as direct effectors of apoptosis (so-called toxic gain of function).

In addition to microglia, astrocytes are also able to produce a number of inflammatory mediators including complement components, cytokines, α-chymotrypsin, prostaglandins, some leukoadhesive molecules (ICAM-1) and some enzymes (iNOS, COX-2), the products of which participate in inflammatory reactions (Akiyama et al., 2000). Astrocytes surround Aβ deposites within neuritic plaques. They are able to release proteoglycanes which inhibit the effect of activated microglia, particularly the phagocytosis of aggregated Aβ (DeWitt et al., 1998).

The third type of brain cells, able to synthesise and secrete inflammatory mediators, are neurones. The brain of patients with AD contains increased levels of these mediators and therefore it is possible that the neurones in AD brain can possibly contribute to their own destruction. Important roles in neurodegeneration and death of neurones in AD are played by pathological shortened forms of tau protein functioning as effectors of apoptosis (Novák et al., 1993, 1999; Fasulo et al., 2000).

Several lines of experimental evidences suggest that many mediators released from activated endothelial cells are also able to participate in the inflammatory processes occuring in the brain (Štvrtinová et al., 1998; Akyiama et al., 2000).

Non-steroidal anti-inflammatory drugs

Until recently, it had been assumed that the target molecules of NSAIDs were represented especially by cyclooxygenases. NSAIDs can, however, regulate also the transcription of a number of inflammatory genes by means of their direct interaction with one group of nuclear transcription factors referred to as PPAR (peroxisome proliferator-activated receptors) (Lehman et al., 1997; Combs et al., 2000). So far, three isoforms of PPAR have been known – PPARα, -γ and -δ. These proteins act as negative nuclear factors via binding with a specific sequence of promoter elements within regulatory genes, thus triggering the transcription of all structural genes with a homologous sequence within the regulatory area. E.g., within monocytes and macrophages, as well as in activated microglia, PPAR-gamma is expressed and its inactive form (lacking the bond with the ligand — agonist) triggers the production of inflammatory cytokines, namely IL-1β, TNF-α, IL-6 and others. The bond with ligand activates PPAR which, in this form, act as antagonists of other transcription factors, especially NF-κB, AP-1 and STAT. By this way, the production of inflammatory cytokines is stopped. All of these three transcription factors activate several dozens of inflammatory genes. Their transmission into the cellular nucleus and thereby also the transcription of appropriate genes are also inhibited by NSAIDs (Ferenčík et al., 1998, 2001).

The natural ligand of PPAR-gamma is represented by prostaglandin J_2 and its intermediate metabolites, as well as by NSAIDs and anti-diabetic drugs of the thiazolidindione group (Combs et al., 2000). All these agonists of PPAR-gamma inhibit the secretion of anti-inflammatory mediators responsible for neurotoxicity and the maintenance of inflammatory reaction, as well as the activation of astrocytes, and the expression of COX-2 in microglia. This proves that the preventive and therapeutic effects of NSAIDs in AD can be more probably based on the impact of PPAR-gamma or other nuclear factors, than that of cyclooxygenase. At the same time, it indicates that the use of further agonists of PPAR-gamma in the therapy of AD can be successful (Kitamura et al., 1999; Combs et al., 2000).

Evidence of damage in neurones inflicted by inflammatory process in AD

Inflammation of the brain or peripheral tissues is nearly always a secondary response to injury of cells or tissue, inflicted by primary pathogens or other injuring insults. It seems that this is valid also in AD, where the primary factors triggering the inflammation are represented by insoluble aggregated forms of Aβ and tau protein, while the stage of dementia and loss of cognitive functions mostly correlate with the occurrence of pathologic forms of tau protein assembled within Alzheimer's pair spiral fibres which are the main component of neurofibrillary tangles, and represent the neural component of senile plaques (Novák, 1997; Novák et al., 1993, 1999).

Even though, the exact mechanism of triggering the injuring inflammatory reaction is not precisely known, it is mostly probable that inflammation is the main cause of neuron injury inflicted in AD. This is proven by the following facts:

1. Many inflammatory mechanisms acting in AD brain are cytotoxic in the periphery. It would be surprising if their effect in the brain would differ.
2. Inflammatory reactions in AD brain are mostly localised in sites, where histologically proven changes are reported (anterior neocortex, limbic cortex), and they are absent or minimal in sites with minor pathologic changes (cerebellum).
3. The occurrence of inflammatory mediators is concentrated into areas surrounding the deposits of Aβ protein and neurofibrillary fibres built up by tau protein.
4. Patients without history of dementia but who nonetheless exhibit sufficient neuritic plaques and neurofibrillary tangles at autopsy to otherwise qualify for the diagnosis of AD show dramatically lower amount of inflammatory markers surrounding the plaques and tangles than AD patients.
5. Several studies prove that the application of conventional anti-inflammatory drugs can delay the onset or slow down the progression of AD.

This possibility has been tested in about 20 clinical studies (McGeer et al., 1996; Hull et al., 1999). The studies most frequently involved the comparison of AD incidence in the group of patients with rheumatoid arthritis requiring a long-term NSAID therapy, with a group of patients at the same age without this treatment. The prevalent majority of studies has proven a significantly higher AD incidence in the group without chronic anti-inflammatory therapy. In Finland, for instance, in 1989, only two patients with

rheumatoid arthritis (RA) died due to AD (0.12 % of the total number of patients), while 0.54 % of patients from the control group of the same size died due to AD (Myllykangas-Lousujarri and Isomaki, 1994). The question as to whether the significantly lower occurrence of AD in these patients with RA is caused either by the anti-inflammatory therapy or by the primary disease, is not clear.

The Baltimore longitudinal study (Stewart et al., 1997) examined 1686 volunteers below 65 years of age, who were regularly treated with NSAIDs. The study discovered that administration of these drugs, for a period shorter than 2 years, reduced the risk of AD by 35 %, while longer administration reduced the risk even by 60 % in comparison with the control group. Further longitudinal studies implied that such positive effect of NSAID pre-medication is brought about in patients below 75 years of age, however the risk of AD occurrence in patients older than 75 years stays unaffected, while NSAID effect is applied not only in patients with RA (Halliday et al., 2000).

The Rotterdam study (Andersen et al., 1995) revealed that only 1.4 % of patients over 55 years of age regularly using NSAIDs suffered from Alzheimer's disease, while 2.5 % of 5,893 patients of the control group without this therapy has developed AD.

McGeer et al. (1996) evaluated four such studies by computer analysis, and discovered that the risk of AD in people who were included into these studies and using NSAIDs, was lower by 52.5 % in comparison with those without this therapy.

Why are NSAIDs mainly tested in the prevention and therapy of AD?

The necessity of these tests implies from the entire current experimental and clinical knowledge (Akiyama et al.– 2000; Halliday et al., 2000) which can be summarised as follows:

1. The inflammatory response of microglia cells is reduced by NSAIDs.
2. COX-2 is constitutively expressed in neurones, but its production is increased in degenerated areas of the brain. This increase can be inhibited by NSAIDs.
3. An increased level of circulating Aβ and increased activation of thrombocytes which synthesise and release Aβ, were discovered in coincidence with AD. NSAIDs are able to decrease the level of Aβ and activation of platelets.
4. NSAIDs antagonise a number of nuclear factors, as e.g. NF-κB or PPAR-gamma, the activity of which, in AD brain, are abnormal in sense of increased transcription of many pro-inflammatory genes (Lukiw et Bazan, 1998; Combs et al., 2000)..
5. Pathological changes in the brain of the aged or experimental animals treated with NSAIDs are significantly smaller than those, not treated and at the same age.

Similar effects, as that of NSAIDs, are discerned also in glucocorticoids. They inhibit the similar production of two key enzymes of prostaglandin production — COX-2 and phospholipase A2, antagonise transcription factors NF-κB, AP-1, decrease the production of many cytokines, ROI, RNI and other inflammatory mediators, inhibit the activation of macrophages and microglia, and the expression of HLA antigens of class II on their surface. On the other hand, their significant adverse effects as immunosuppression, infertility and neurodegenerative changes appearing especially in coincidence with long-term administration, make them less advantageous for such application.

The adverse effects, however, also appear in coincidence with NSAID administration. They imply especially from the inhibition of constitutive COX-1, the products of which are needed in normal function of the gastric mucosa, kidneys and thrombocytes. The preparations which preferably inhibit the inflammatory COX-2 and affect COX-1 to a negligible extent or not at all, do not have these adverse effects, the fact of which makes them especially advantageous in long-term application (Vane et Botting, 1998; Marnett et Kalkutkar, 1999).

NSAIDs generally implies: the lower their selectivity for COX-2, the higher is their gastrointestinal toxicity. On the basis of this rule assessed according to clinical and experimental investigations, Mitchell et Warner (1999) have divided NSAIDs into three groups:

1. Preparations with most severe adverse effects on the gastrointestinal tract: ketorolac, ketoprofen, indomethacine, tolmetin and piroxicam.
2. Preparations with mild adverse effects: acetylsalicylic acid, fenoprofen, naproxen, and sulindac.
3. Preparations with relatively little adverse effects: ibuprofen, diclofenac and diflunisal.

Frölich (1996) has divided NSAIDs according to their pharmacodynamic effect and the ability to inhibit individual isoenzymes COX-1 and COX-2 into four groups:

1. Selective inhibitors of COX-1: low-dose acetylsalicylic acid (daily dose to 325 mg).
2. Non-selective inhibitors of COX-1 and COX-2: all classical NSAIDs, from high-dose acetylsalicylic acid through ibuprofen, diclofenac to piroxicam.
3. Selective inhibitors of COX-2: etodolac, nimesulid, meloxicam.
4. Supraselective (specific) inhibitors of COX-2: rofecoxib, celecoxib.

The lowest occurrence of adverse effects is proven in selective and specific inhibitors of COX-2, the development of which is currently under intensive investigation (Vane et Botting, 1998; Mitchell et Warner, 1999). The effect of these NSAIDs was proven not only in the prevention of AD (Blan et al., 2000), but also in the treatment of pain and inflammation in osteoarthrosis and rheumatoid arthritis, and in the therapy of acute non-tumour pain (Lipsky, 1999; Clemett et Goa, 2000). Both types of these inhibitors dispose with better gastrointestinal protective and tolerance profiles in comparison with non-selective NSAIDs. Their use in rheumatology has become a fact, and the proof of their effect in the prevention of AD and some tumour diseases is currently under investigation. The current clinical practise uses two specific inhibitors of COX-2: celecoxib (Celebrex™) (Geiss, 1999) introduced into the USA market by Searle and Pfizer at the end of 1998, and rofexocib (Vioxx™) (Chan et al., 1999) produced by Merck Sharp and Dohme. Celebrex was rewarded as the Drug of the Year 2000. Both drugs are registered in the Slovak Republic — celecoxib has been registered in therapy of rheumatoid arthritis and osteoarthrosis since 1999, and rofecoxib has been registered in the therapy of osteoarthrosis since 2000 (Špringer et Chalabala, 2000). Both of them have a comparable anti-inflammatory effect, but rofecoxib exceeds celecoxib by its analgesic effect (Malstrom et al., 1999).

The recent studies have revealed that selective and specific inhibitors of COX-2 are effective in the prevention of some tumour diseases as colorectal carcinoma, carcinomas of the skin, breast and prostate.

The results of experimental studies performed on animals give us the hint that these preparations dispose with an anti-tumour effect, and some have an anti-neoplastic effect. The patients with familial adenomatous polyposis which is considered to represent a significant pre-cancerous stage of colorectal carcinoma, show a reduced number of colorectal polyps after a continuous six-month treatment with celecoxib in the daily dose of 2x400 mg (Steinbach et al., 2000). An important fact has been discovered, namely that the administration of celecoxib in patients with bronchial asthma and intolerance to acetylsalicylic acid and other NSAIDs, does not bring about bronchospasms which are observed after administration of other NSAIDs (Dahlen et al., 2001).

Conclusion and other perspectives

The basic question as to whether the inflammatory response to Aβ and tau protein is primarily responsible for the origin of AD, or if it represents only a consequence of other etiopathogenic mechanisms, has not been precisely answered so far. Despite this fact, it is currently generally accepted that the inflammatory process has a significant role in the pathogenesis of AD.

A great number of experimental studies confirm that the course of inflammatory reactions within the brain does not differ significantly from that in the periphery. The cells of the brain are able to synthesise locally practically all inflammatory mediators, but these cells differ from those in the periphery. The transmigration of typical inflammatory cells from the periphery to the brain tissue is restricted by the blood-brain barrier. Despite the fact that in some situations it does not have to be fully impermeable, it is not assumed that neutrophils and inflammatory T-lymphocytes from circulation participate in inflammatory processes within the brain significantly.

The activating effect of inflammatory mediators on the cells of the macrophage lineage, as microglia, seems to be similar to that in the periphery. However, very little is known about how these mediators affect the neurones. For instance, if a neuron secretes complement components, does it contributes, in this way, to its own destruction, or does the role of the complement in the brain differ from that in the periphery? If so, what role is it? Similarly, the alarm cytokine TNF-α has a cytotoxic effect in the periphery, while in the brain it can have a cytoprotective effect. These examples may be sporadic, or are there more of them? These questions will have to be answered in order to accomplish full understanding of the function of inflammatory mediators within the brain.

It seems that despite the similar damaging effect as in the periphery, the inflammatory process within the brain can have particular differences which will have to be precisely known. For instance, in AD it obviously involves a long-term process of moderate intensity, which starts to manifest only in a particular stage. Is this the result of the cumulative growth of inflammatory changes, or does it imply from the engagement of other factors coinciding e.g. with age? The perception of these differences can help in revealing the new pathogenic associations, more precise diagnosis, prevention and therapy not only in AD, but also in other CNS diseases. Brain inflammatory markers are present also in Parkinson's disease, the complement can participate in the injury of myelin in multiple sclerosis. For a long period of time it had been assumed that the inflammatory component did not participate in prion diseases as Creutzfeldt-Jacob disease, but the current understanding is changing (Akiyama et al., 2000). Therefore, it is possible, that inflammation could represent a part of pathogenic mechanisms in many of neurodegenerative damages, or probably in all of them.

The group of known risk factors contributing to the onset of AD and other neurodegenerative diseases with inflammation, will have to be widened by "a phenotype of inflammatory response" which predisposes individuals to the origin of AD (Bales et al., 2000). It has been discovered, that several inflammatory cytokines (e.g. IL-1, IL-6) are polymorphous. They are encoded by several alleles, the products of which (isotypes of individual cytokines) do not respond with the same readiness and intensity to particular inflammatory stimuli. This means that an individual who owns alleles which are "readier to inflammation" will be more likely to develop AD than the individual whose alleles express "less ready" cytokines. In this way, besides the known genetic predisposition implying from the presence of allele for apoE4, it will be necessary to include also some alleles for inflammatory cytokines.

Despite several, so far unanswered questions, it is necessary to count with inflammation as the key pathogenic mechanism in the onset and development of AD. Anti-inflammatory drugs could therefore significantly delay the onset and slow down AD development, particularly at older age (55—75 years of age). Long-term application of non-steroidal anti-inflammatory drugs selectively inhibiting cyclooxygenase 2 can be considered as mostly perspective. According to current knowledge, these drugs are sufficiently effective since they restrict inflammatory manifestations on various levels, and have no particular adverse effects.

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