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Review article

Perivascular drainage of amyloid beta (Aβ) and waste products from the brain and its failure.

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Abstract

The disorder of intracerebral accumulated amyloid beta (Aβ) drainage, as well as the drainage of various waste products related to brain metabolism and permanent biochemical reactions, lead to brain homeostasis disorder, the breakdown of important regulatory functions and to the occurrence of cerebral amyloid angiopathy and rapid development of genetically programmed Alzheimer's disease (AD). The brain does not have its own standard lymphatic networks and therefore this important role is mostly taken over by the perivascular system which is located in the areas within the basement membranes of capillaries, arterioles and arteries. The driving force of this drainage is not formed by previously stated forces that are conditioned by heart functioning through the accompanying arterial pulsations, but by the newly discovered internal force of arterial and arteriolar walls, the so-called vasomotion. Moving in the direction opposite from the direction of the blood stream and pulse wave, these pulsating forces are based on rhythmic intracellular oscillations of Ca^{2+} ion concentration in vascular smooth muscle cells. With the participation of a number of molecular components, these oscillations cause the vasomotion phenomenon and their disturbance leads to perivascular drainage alterations and severe complications. This paper gives us a thorough analysis of crucial events that lead to the perivascular system alterations.

KEY WORDS: Alzheimer's disease, cerebral amyloid angiopathy, perivascular drainage, hydroxyl radicals, oxidative stress

Introduction

Alteration of the perivascular drainage from the brain

A number of complex studies related to the etiology of Alzheimer's disease (AD), the severe, chronic, and lethal neurodegenerative phenomenon, increasingly indicate the enormous importance of the regular drainage from the brain. This drainage refers to the Aβ peptide produced in the brain, many metabolic waste products, as well as numerous waste products resulting from the vitally important biochemical processes. Not entering into the detailed pathophysiology of AD, this disease, with its polygenetic etiology, is in fact the result of the elevated neuronal production of amyloid beta (Aβ) peptide, its enormous extracellular cerebral accumulation, and altered drainage from the cerebral parenchyma. Along with the mentioned problem connected with Aβ, it is also important to emphasize the elevated intracerebral accumulation of transition metals in AD, especially iron ions, the accompanying oxidative stress, as well as their weakened drainage out from the brain (***Fig. 1,2***). Investigations into cerebral physiology, pathophysiology, and pathology, indicate a lot of important Aβ and superfluous iron ions drainage pathways. The crucial factor which determines the analysis of the perivascular lymphatic brain drainage, connected with the basement membranes (BMs) of the arterial, arteriolar, and capillary walls interior, is the specificity of the brain, which does not have a standard lymphatic drainage net like other body organs. The estimated value of the drainage rate of the brain is in the range of 0.11-0.29 μL/min/g (data obtained from rat brain by combining histological and mathematical analyses). Some considerably investigated nonconventional brain lymphatic drainage pathways are: drainage through the blood brain barrier (BBB), blood cerebrospinal fluid barrier (BCSFB), choroid plexus, arachnoid granulations (AGs), paravascular drainage (connected with the cerebral vasculature surfaces), perineural drainage, lymphatic drainage through the orbits into the sinus sagitalis superior, and nasal cavity (lymph inflow along the

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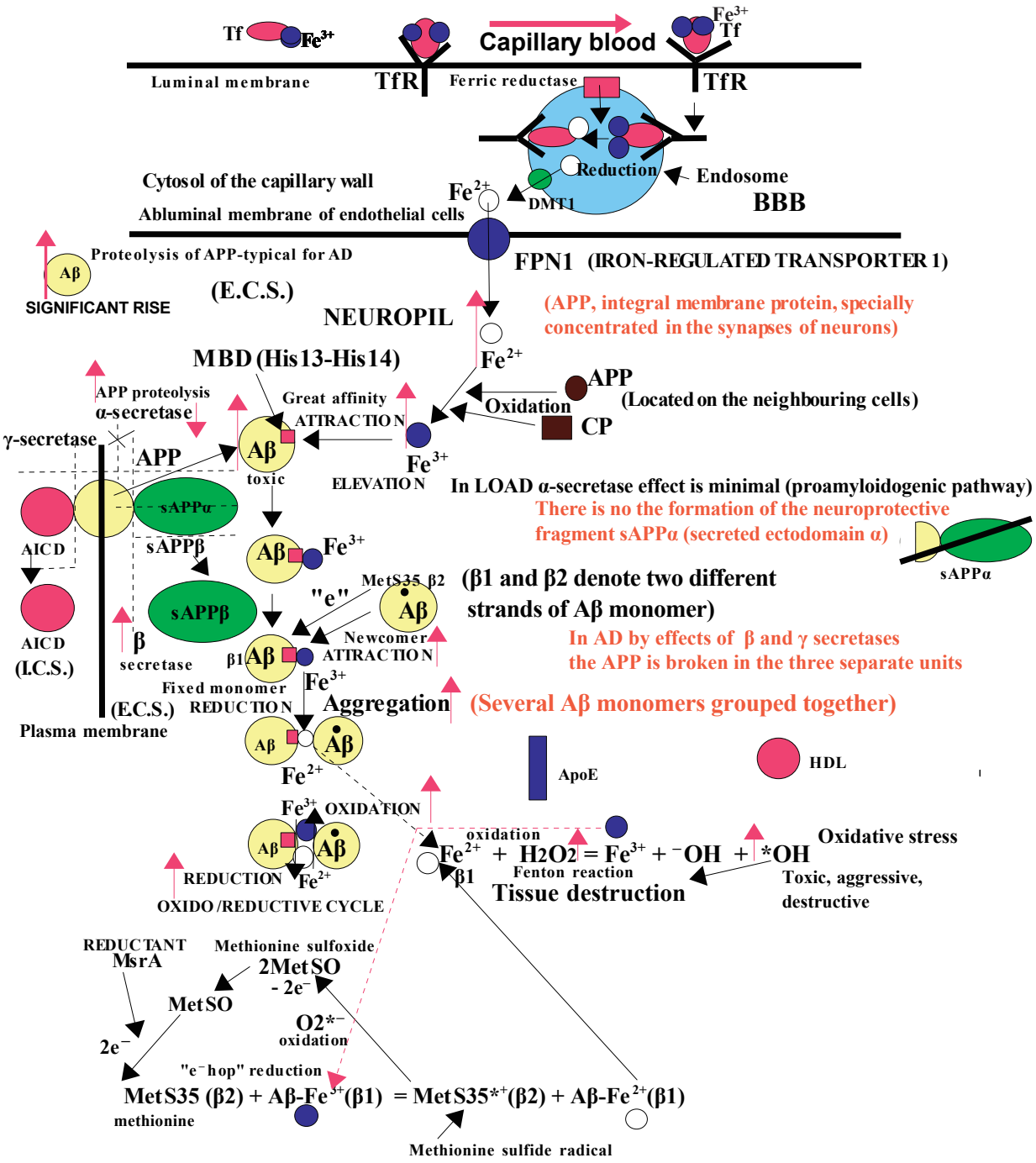

Fig. 1. **Schematic presentation of events connected with Aβ and Fe^{3+} elevated intracranial values.**

Oxidation, loss of electrons; reduction, gain of electrons; hydroxyl radical (*OH), neutral form of the hydroxide ion (OH^-), highly reactive, aggressive and short lived; MsrA, peptide, methionine sulfoxide reductase, repair enzyme for proteins inactivated by oxidation; MetS35, methionine, one of nine essential amino acids; Fe^{3+}, ferric iron; Fe^{2+}, ferrous iron; H_2O_2, oxidizer, the simplest peroxide; CP, ceruloplasmin, a ferroxidase enzyme; Aβ, amyloid beta; APP, amyloid precursor protein; ApoE, apolipoprotein E; Tf, transferrin; TfR, Tf receptor; DMT1, divalent metal ion transporter-1; MBD, metal binding domain; FPN1, ferroportin 1, iron-regulated transporter 1; BBB, blood brain barrier; E.C.S., extracellular space; I.C.S., intracellular space; AD, Alzheimer's disease ; LOAD, late onset AD; HDL, high-density lipoprotein; AICD, APP intracellular domain; sAPPα, secreted ectodomain α: sAPPβ, secreted ectodomain β; CTFB, membrane bound fraction;.

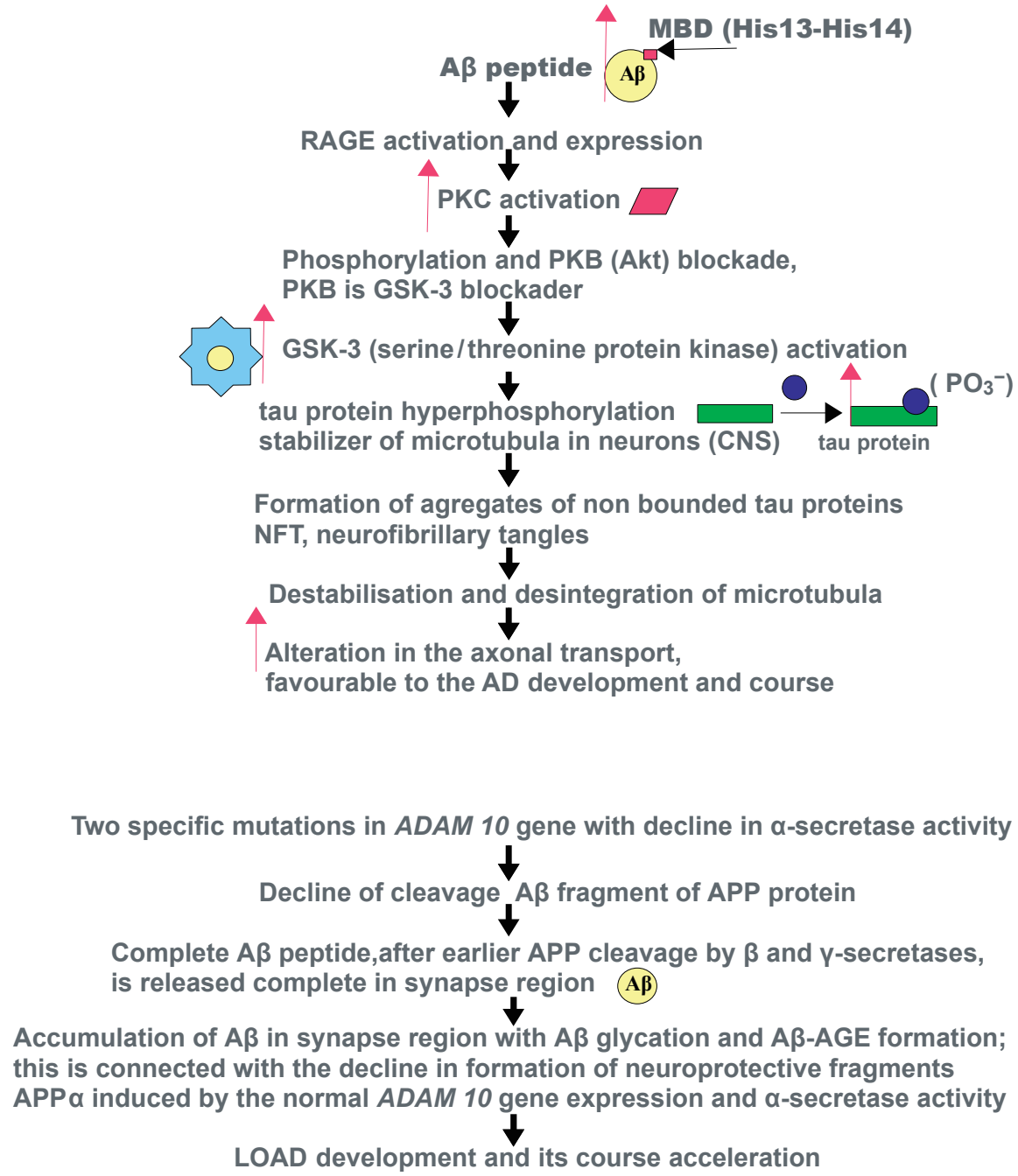

Fig. 2. **Schematic presentation of events induced by RAGE activation and *ADAM 10* gene mutations.**

Aβ, amyloid beta; APP, amyloid precursor protein; AGEs, advanced glycation end products; RAGE, receptor for AGEs; glycation, non-enzymatic glycosylation, covalent attachment of sugar to protein; MBD, metal binding domain; PKC, protein kinase C, phosphorylation, chemical addition of a phosphoryl group (PO_3^-) to an organic molecule; PKB (Akt), serine/threonine-specific protein kinase; GSK-3, glycogen synthase kinase; NFT, neurofibrillary tangles; tau-protein, protein that stabilizes microtubules in neurons; AD, Alzheimer's disease; LOAD, late onset AD; APPα, secreted ectodomain α; CNS, central nervous system.

olfactory tract-tractus olfactorius). In addition, it is also necessary to emphasize the processes of Aβ intracellular (proteasome and ubiquitin-proteasome route, lysosomal cathepsin enzymes thiolmetalloendopeptidases), and Aβ extracellular degradation (neprilysin, matrix-metalloproteinases 2, 3 and 9). However, this is not the theme of this study. Perivascular drainage, connected with the walls of cerebral vasculature, is increasingly the subject of intensified investigations. This drainage, located in the BMs of the leptomeningeal and cortical arteries, and in the BMs of their terminal branches, arterioles and capillaries, driven by vasomotion forces, presents the crucial system of brain homeostasis, and its failure leads to the occurrence of cerebral amyloid angiopathy (CAA), earlier onset of AD, and its accelerated course (***Fig. 2, 3***) [1-6].

Perivascular drainage pathway

Before the analysis related to the complex pathophysiology of the development of perivascular drainage alterations, the occurrence of CAA, and the onset and acceleration of the course of AD, it is necessary to present the general characteristics of this crucially important pathway. The starting-point of this pathway is located on the walls, or the BMs of cerebral capillaries. Molecules of Aβ and other waste material first pass from the subarachnoid space (SAS), where it has previously entered, and then across the very permeable pia, into the paravascular space, located between the pia and the BM of the glia limitans. After that they pass across the BMs of glia limitans (passing through the astrocytic endfeet clefts and AQP4 channels), and finally enter into the brain parenchyma, or travel further on distally along the mentioned space up to the capillary level and here enter into their BM.

One portion of Aβ and other waste products travel further along the markedly narrow paravascular space, and at the parenchyma level pass through the astrocytic endfeet clefts and AQP4 channels, and enter into the parenchyma, while one part, moving distally, at the level of SAS, enters across the permeable pia into its formerly abandoned space (SAS). In fact, the movement is circular. From SAS, the arriving Aβ and remaining waste can enter into the AGs and through them into the superior sagittal sinus (SSS), and further on, by venous blood into the venous body system and heart. A part of the Aβ and waste products enters the capillary wall interior and its BMs, then into the cortical and leptomeningeal artery media, where they move along their BMs between vascular smooth muscle cells (VSMCs) in opposition to the direction of blood flow. The driving force for this movement is not dependent on arterial pulsations, or on pulse pressure, but exclusively on the vasomotion, *i.e.* on its vasomotion waves. There are a number of written articles analyzing the mechanism of this movement with the domination of events linked with actin, myosin, and Ca^{2+} oscillations in the VSMCs. In order to understand the above presented facts, it is necessary to emphasize that Aβ is generated in the brain parenchyma, by the proteolysis of the transmembrane protein APP (amyloid precursor protein) located on neuronal cell membranes. From there, Aβ goes on, passing through the BMs of glia limitans, the paravascular space, and pia, then enters the SAS, and continues its circular pathway, as it has been described earlier in the text (***Figs. 3-5***) [4-9].

Basement membrane (BM) structure

The analysis of the BM by electron microscope shows that it is composed of two layers (width about 50 μm) (***Figs. 4, 5***). The layer closer to the endothelium is the basal lamina (BL, basal membrane, tunica intima-width 20-100 nm), and the deeper layer connected with the previous one forms the reticular connective tissue (lamina reticularis). Both these two layers are connected by collagen VII molecular fibrils and fibrillin microfibrils. BL is composed of the superficially layer lamina lucida and the deeper layer, located along the reticular layer of lamina reticularis, named lamina densa. Lamina densa is 30-100 nm wide, and composed of the collagen IV fibril net and the sulfate rich proteoglycan perlecan (BM-specific heparan sulfate proteoglycan, which cross links many extracellular matrix components-collagen, enzymes, glycoproteins). Lamina lucida is about 60 nm wide and somewhat more luminous, and contains a lot of laminins, integrins, entactins and dystroglycans. The main components are laminins (high-molecular weight protein of extracellular matrix, the major component of the BL). These heterotrimeric glycoproteins (laminins) have connective places for integrins, type IV collagen and heparan sulfate. Their important function is the formation of the structural net connected with the collagen IV net. Lamina reticularis is built from the reticular fibers net composed of collagen type III (homotrimer-protein composed of three long chains). Fibers are synthetized by reticular cells. Due to the VSMCs location (capillary BM does not have these cells) in this layer, and due to their role in vasomotion wave's generation, it is evident that perivascular drainage takes place along this layer.

The essential substance of the lamina reticularis is the poroelastic extracellular material and the fluid component, *i.e.* interstitial fluid (ISF), which is primarily water. Besides collagen, the other important components of the arterial wall are elastin molecules grouped in thin concentric porous plates. Each of these laminas is connected with the concentric ring of VSMCs forming the flexible lamellar unit of the arterial wall [10]. BMs are becoming increasingly important in biology [4]. According to the most recent research, the BMs take part in blood filtration, muscle homeostasis, the storing of growth factors, the cytokine control of angiogenesis, and tumor growth. Their functions are extensively presented by Moris AW *et al.* [11], Pozzi A *et al.* [12], and Hawkes CA *et al.* [13]. The BL in the CNS is the product of vascular endothelial cells secretion (continuous endothelial lining). It is visible only by electron microscope. Besides the supporting function to the neighboring cells, the BMs are also an important storage site for growth factors and take part in physiological processes in these cells. It is especially important to emphasize their role as the main components of the perivascular drainage pathway (IPAD, intra-mural-peri-arterial drainage) (***Figs. 4, 5***).

Perivascular drainage alterations in AD

The analysis of the alterations of the perivascular Aβ drainage of iron ions, as well as the harmful waste products resulting from a number of biochemical processes in the brain, crucial for the onset and course of AD, primarily requires an insight into the complex morphological and

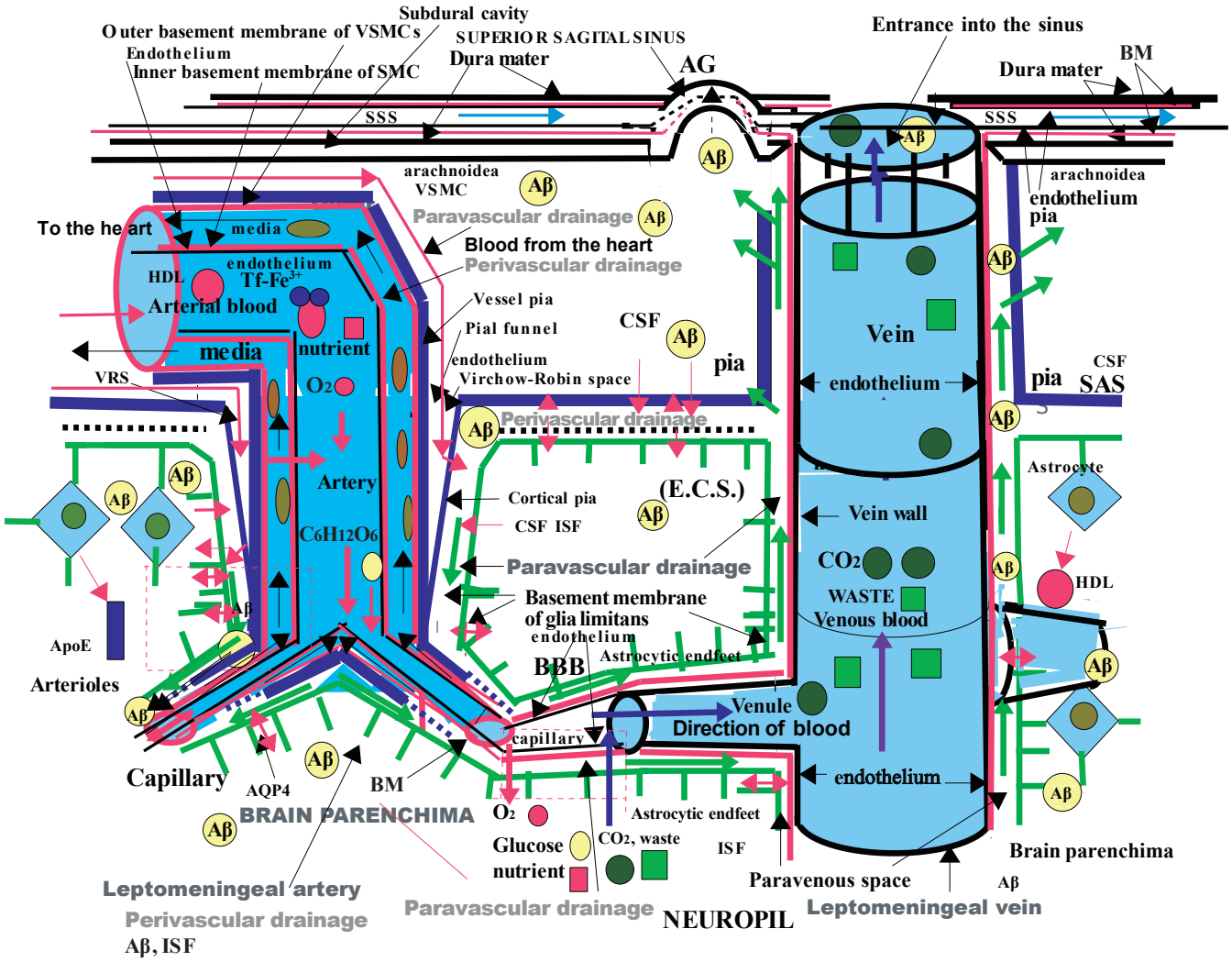

Fig. 3. **Schematic presentation of the Aβ, iron, and waste products drainage from the brain.**

BBB, blood brain barrier; this figure represents the additional survey of Figure 3 in Reference 5), On the left side of the figure a rectangle limited by small red lines is visible-it in fact presents **Fig. 5**. The other rectangle including capillaries presents **Fig. 4**. Basement membrane (BM) at the capillary level is the result of the proximal integration of the outer and inner BM of VSMCs. VSMC, vascular smooth muscle cell; SSS, superior sagital sinus; HDL, high-density lipoprotein; CSF, cerebrospinal fluid; E.C.S., extracellular space; Aβ, amyloid beta; VRS, Virchow-Robin space; O_2, oxygen; $C_6H_{12}O_6$, glucose; CO_2, carbon dioxide; ISF, interstitial fluid; SMC, smooth muscle cell; AQP4, aquaporin 4, channel located on astrocytic endfeet, important for Aβ drainage; SAS, subarachnoid space; AG, arachnoid granulation; Tf-Fe^{3+}, transferrin-ferric ion complex; ApoE, apolipoprotein E.

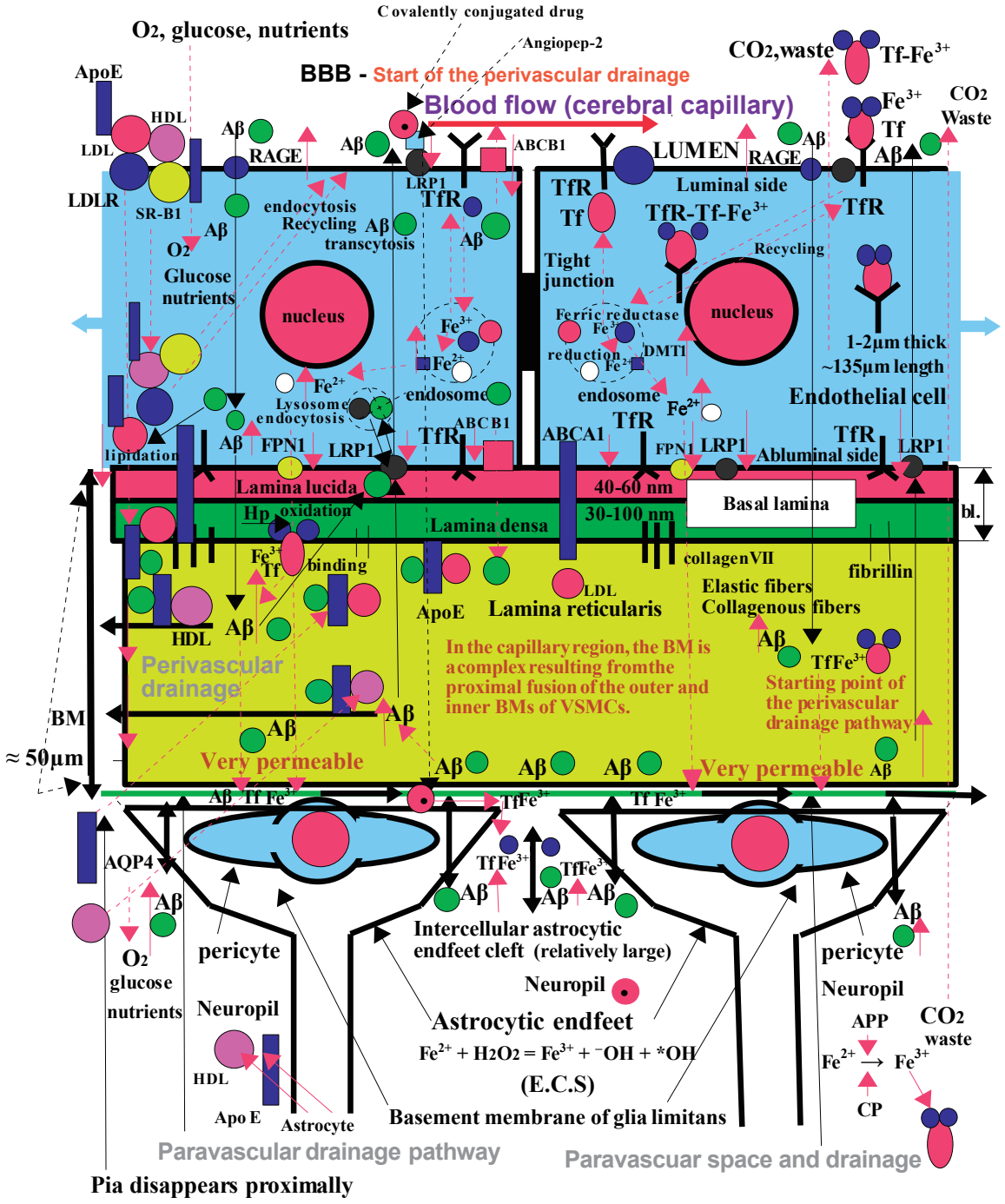

Fig. 4. **Structure of the cerebral capillary basement membrane: effects of aging and AD (longitudinal cross-section).**

In the upper part of the figure two endothelial cells connected by a tight junction are visible. Above the luminal side of both cells, the red arrow denotes the capillary lumen, anterograde blood flow direction, some receptors (TfR, RAGE) and Aβ peptide molecules. Below the cells the basement membrane is visible which is composed of two layers, the basal lamina (bl.) and lamina reticularis. The basal lamina is composed of the lamina lucida (below the endothelium) and lamina densa; Hp, hephaestin, a transmembrane copper-dependent ferroxidase, functionally closely bound with the FPN1 receptor, responsible for the oxidation and transport of Fe^{2+} from the cell interior across its abluminal cell membrane. The obtained Fe^{3+} binds promptly with the locally present Tf ; Angiopep2, conjugated drug; AD, Alzheimer's disease; Aβ, amyloid beta; APP, amyloid precursor protein; CP, ceruloplasmin; E.C.S., extracellular space; AGEs, advanced glycation end products; RAGE, receptor for AGEs; LDL, low-density lipoprotein; HDL, high-density lipoprotein; LDLR, LDL receptor; ApoE, apolipoprotein E; BBB, blood brain barrier; Tf, transferrin; TfR, Tf receptor; ABCA1, ATP-binding cassette transporter 1; ABCB1, ATP-binding cassette sub-family B member 1; FPN1, ferroportin 1; LRP1, low density lipoprotein receptor-related protein 1; DMT1, divalent metal ion transporter-1; VSMCs, vascular smooth muscle cells; BM, basement membrane; AQP4, aquaporin 4.

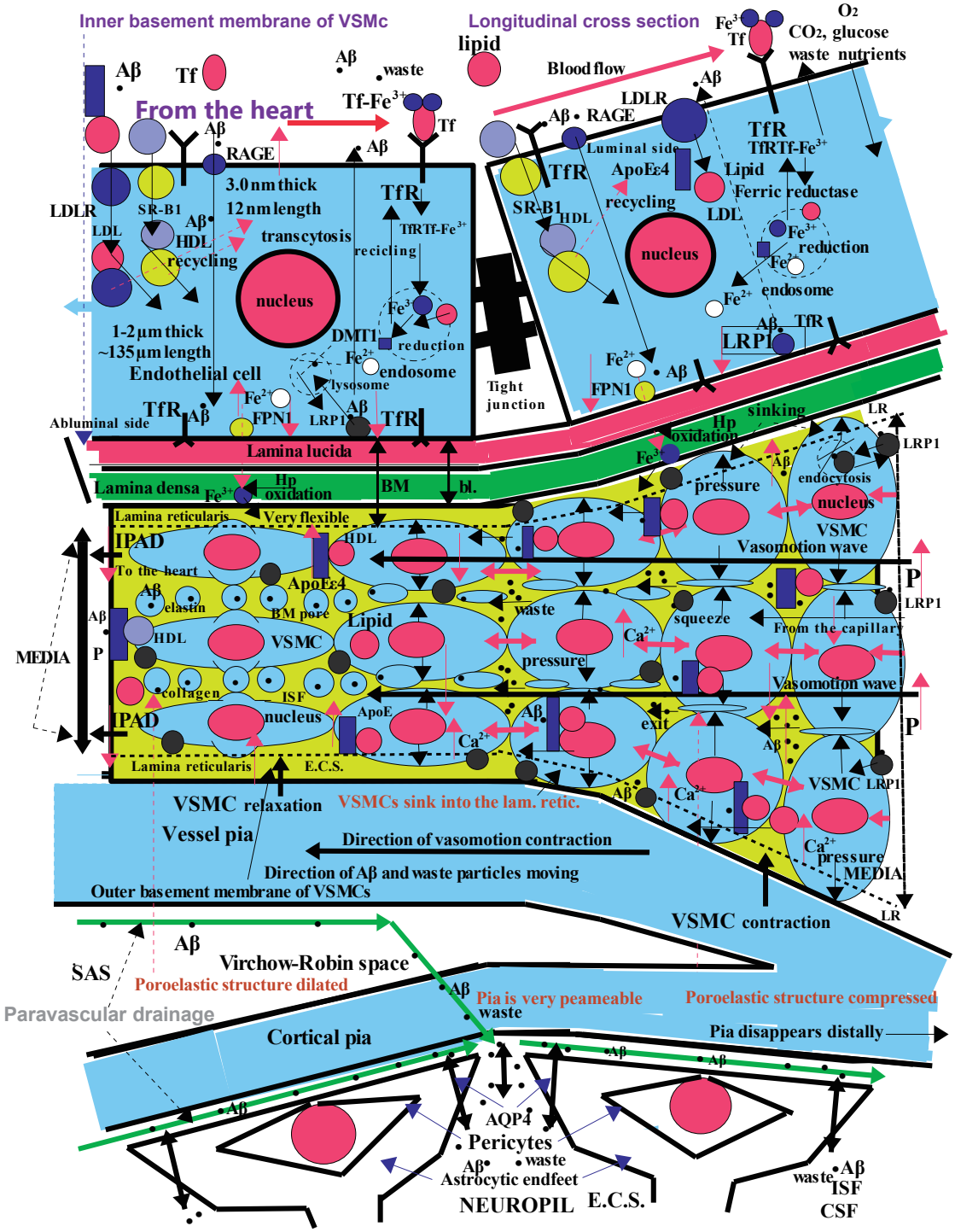

Fig. 5. **Schematic presentation of intra-mural peri-arterial drainage (IPAD)**

The LRP1/Aβ endocytosis = IPAD pathway is visible; VSMC, vascular smooth muscle cell; AQP4, aquaporin 4; Tf, transferrin; TfR, Tf receptor; Hp, hephaestin; AGEs, advanced glycation end products; RAGE, receptor for AGEs; CSF, cerebrospinal fluid; ISF, interstitial fluid; BBB, blood brain barrier; Aβ, amyloid beta; APP, amyloid precursor protein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; LDLR, LDL receptor; ApoE, apolipoprotein E; VSMC, vascular smooth muscle cell; LRP1, low density lipoprotein receptor-related protein 1; SR-B1, scavenger receptor class B member 1; FPN1, ferroportin 1; DMT1, divalent metal ion transporter-1; SAS, subarachnoid space; E.C.S., extracellular space;BM, basement membrane; bl., Basal lamina; LR, lipoprotein receptor.

functional changes of the cerebral vasculature connected with the aging process, but without the presence of active AD. Perivascular drainage alterations during normal aging (also with the presence of AD), manifests itself with Aβ aggregation in the middle layer of leptomeningeal artery walls (media), *i.e.* in the BMs which envelop VSMCs, as well as in the BMs of their final elements, arterioles and capillaries. The occurrence of this aggregation is defined as CAA. It is important to emphasize that it occurs in 30% of aged people without any sign of AD, and in 90% with AD. This study does not deal with the complex etiology of AD, nor with the analysis of the whole complex of genes important for the onset and course of this severe, chronic, and lethal neurodegenerative disease; it is primarily involved in the problem of perivascular drainage, connected with the BMs of the mentioned vessels, whose alteration has a crucial role in AD pathophysiology, and consequently in the development and course of the clinical picture and the final lethal result of the disease. Before further analysis, it is important to emphasize that AD occurs in two essential forms: the early form or EOAD (early onset AD) before 65 years, about 5% of all cases, and the late form or LOAD (late onset AD), 65 years and more, about 95% of all cases. The clinical picture of both forms is practically the same. The crucial signs of the disease are progressive loss of memory, loss of speech, and failure of cognitive functions. The course of the disease is marked with increased weakness of the patient, who at the end is completely disabled and dependent on someone else's help and nursing [5-7].

Now it is necessary to concentrate our attention to the arteries and their terminal units (arterioles and capillaries) in the aging process without the presence of AD. The investigation of the perivascular drainage starting point structure, *i.e.* capillary BMs of aged human brains, shows their vacuolization, reduplication, and thickening. The investigation of the capillary BM width in mice aged 23 months, by transmission electron microscopy, has shown a significant increase in the width (thickness in nm) of their cortex BMs, hippocampus, and thalamus, as compared to mice aged 2 and 7 months (2 months: 65.79, 62.57, 70.11 nm; 23 months: 97.49, 109.50, 108.43 nm). In mice aged 23 months, in cortical BMs, there is a significant drop in the amount of collagen IV, laminin, and nidogen 2. At the same time, in these mice, there is a significant increase of N-terminal fibronectin fragment levels, as well as an increase of perlecan levels. Collagen IV, laminins, and nidogens are all markedly defined inhibitors of Aβ aggregation and destabilizers of previously formed Aβ fibrils. Heparan sulfate proteoglycans (HSPGs), *i.e.* agrin and perlecan, accelerate the mentioned aggregation. The presented data show changes in the cerebral vasculature BMs connected with aging, which are favorable for the development of the proamyloidogenic environment. Again, it is necessary to emphasize that thickening, cleavage, and duplication, as well as the occurrence of abnormal inclusions of cerebrovascular BMs, is a frequent phenomenon in the brain of aged humans and animals, and that this phenomenon is much more visible in the brains affected by AD (***Table 1***) [10, 13-21].

Further in the discussion, it is necessary to point out that in the presented figures, close to receptors, up and down directed arrows are visible. Their direction indicates the receptors activity, *i.e.* their expression, and is related to the decline in magnitude of perivascular drainage. The drainage decline is closely linked with increasing age. It is very instructive to present the example of LRP1 receptor (low density lipoprotein receptor-related protein1, receptor mediated endocytosis of Aβ and its effective drainage from the brain) located at the abluminal side of the endothelial cells (BBB). Experiments with laboratory mice show a significant difference in LRP1 expression (significant drop in its expression and effectiveness) between 34 month old mice and 3 month old mice (***Fig. 4***) [22].

Vascular alteration with aging

In their earlier work, Hawkes CA *et al.*[13] present findings related to the Aβ deposition in the walls of leptomeningeal and cortical arteries in form of CAA in the normal aging process, and its accelerated rate in AD. They declare that the Aβ deposits in the cerebral vasculatory can be observed in nearly 30-40% of aged individuals without any sign of dementia. This percent is much higher in demented persons. The Aβ deposition is accompanied by capillary thickening, vessel tortuosity, vasoconstriction, angiogenesis inhibition, and pericyte, endothelial cell, and VSMC death. During the aging process, the enzymatic degradation, Aβ absorption in the blood, as well as its influx into the microglia, are also diminished or disturbed. The already mentioned compounds, the integral components of the vasculature, laminin, collagen IV, and nidogen by direct effect on Aβ, obstruct its aggregation and favor the dissolution of already formed fibrils. On the other hand, agrin and perlecan (both are HSPGs) induce Aβ fibrillization and the stabilization of already formed fibrils. All presented changes, in fact linked with the course of normal aging, lead to the dysfunction of the perivascular drainage and induce Aβ deposition. The authors emphasize their own experimental results related to dextran molecule drainage by hippocampal microvasculature. Mice aged 22 months have an evidently weaker drainage compared to mice aged 3 and 7 months. They have found evident differences in the capillary structure thickness and in the protein composition of their walls. The subsequent paper by Hawkes CA *et al.*[19] published two years later, confirms these findings.

Keable A *et al.*[23], show the disturbance of soluble Aβ perivascular drainage from the brain induced by age. By the use of Image J (a Java-based image processing-National Institutes of Health and Laboratory for Optical and computational Instrumentation, LOCI, University of Wisconsin) and confocal microscopy (optical imaging technique for increasing optical resolution and contrast of a micrograph), the authors analyze the immunostaining paraffin sections of post-mortem human occipital cortex, and find a marked elevation of nidogen 2 (enactin) parallel with aging. In CAA, its level is markedly reduced. By aging, arteries become increasingly stiffer and rigid and lose their innervation. The authors continually emphasize the biochemical changes of the cerebrovascular BMs which go along with the aging process. The Aβ aggregation in the vascular walls of aged persons without symptoms and signs of dementia, strongly indicates the aging induced disturbance of Aβ elimination from the brain. Through time, this leads to the occurrence of CAA, as is in the case of AD.

ZekonyteJ *etal.*[24] in their study related to the dynamics of perivascular drainage, emphasize the great importance

Table 1.
Structural changes of basement membranes related to "normal" aging without any signs of Alzheimer's disease (AD).

Different parameters
Cappilary basement membranes:
Vacuolization
Reduplication
Thickening
Cleavage
Collagen IV – drop ↓ → Amyloid beta (Aβ) aggregation inhibition and destabilization
Laminin – drop ↓ → of early formed Aβ fibrils;
Nidogen 2 (enactin) elevation (in the case of present CAA clear drop)
N-terminal fibronectin fragment – elevation ↑
Perlecan – elevation ↑ → accelerate Aβ
Aggregation and HSPG Agrin → its fibrilization
Aβ sedimentation in the form of CAA – elevation ↑
30-40% elderly subjects without AD have CAA;
Besides capillary thickening, cerebral arteries show tortuosity, vasoconstriction, inhibition of
angiogenesis, death of pericytes, endothelial cells, and VSMCs;
It is also visible enzymatic Aβ degradation, drop of the Aβ absorption ↓ into the blood,
and drop of its influx ↓ into microglia;
Arteries with aging loss their elasticity, become stiffer and loss inervation.

The data on the table are related to the essential events connected with the aging of brain structures and functions; the data are not influenced by AD; to obtain this data, among different methods, transmission electron microscopy was primarily used; collagen IV, a type of collagen primarily located in the basal lamina, structural protein; laminin, high- molecular weight protein located in the extracellular matrix of basal lamina, it is biologically very active; nidogen 2, enactin, protein located in basal lamina, biologically very active; N- terminal fibronectin fragment, some different functiions are mentioned, effects in the mononuclear phagocyte function, inhibition of fibronectin to fibroblasts, inhibition of platelet aggregation; perlecan, heparin sulfate proteoglycan 2, included in binding and cross-linking of many extracellular matrix components and cell surface molecules, primarily are located in the extracellular matrix of basement membranes; HSPG agrin, heparin sulfate proteoglycan, important for the structure and function of the neuromuscular junction, it binds with many other proteins; VSMCs, vascular smooth muscle cells; Aβ, amyloid beta; AD, Alzheimer's disease; CAA, cerebral amyloid angiopathy.

of continuous and effective Aβ elimination from the brain. The accumulation of this peptide, regardless of the presence of AD, permanently occurs in the brain parenchyma and its vasculature, especially in the carriers of *APOEε4* allele. The authors relate the disturbance of this drainage to the reduction of pulse waves linked with the aging process of arteries. As distinguished from a number of recent researchers, among them, especially Aldea R *et al.*[4], who declare that pulse waves, do not have any effect on the perivascular, but only on the paravascular drainage, Zekonyte J *et al.*[24] give great importance to the, until now, generally current attitude emphasizing the crucial role of pulse waves, especially reflexion waves. According to them, the reduced strength of linking ApoEε4/Aβ complex to the BM structures of the vascular wall (laminin), as related to the evidently stronger linking of the mentioned complex with the other two *APOE* alleles (ε2 and ε3), makes possible its oscillations during the passing of pulse waves and in this way, the decline of the drainage intensity. However, according to a number of recent researches, the drainage process still depends on a completely different mechanism (see Aldea R *et al.*[4]). The most recent experiments indicate the crucial importance of the system of cerebral arteries and arterioles BMs and among them, the embeded VSMCs.

The role of ApoE

The *APOEε4* gene codes the ApoEε4 protein. In the central nervous system (CSN), ApoEε4 protein synthesis is established in astrocytes and microglia (astrocytes as part of the BBB surround cerebral capillaries and secrete ApoE proteins into the narrow paravascular space). In

relation to the other two alleles, ε2 and ε3, ApoEε4 is a strong risk factor for the occurrence of AD. It is established that ApoEε4 is an amphipathic peptide whose N-terminus carries two hydrophilic groups of residues (res. 55-79 and res. 140-158), and C-terminus which carries hydrophobic residues 261-287. In the perivascular space, this allele is significantly less effective in the events related to Aβ drainage. The drainage process is otherwise induced by the action of the ABCA1 (ATP-binding cassette transporter1, cholesterol efflux regulatory protein, CERP), located on the abluminal side of the cerebral endothelial cells. It leads to the lipidation (joining with the lipid component) of the ApoE protein with the formation of lipoprotein particles. The investigations show that the lipidation of ApoEε4 lipoprotein is significantly weaker as related to ApoEε2 and ApoEε3. ApoEε4-HDL (high-density lipoprotein) particles are markedly smaller than the other two alleles. Otherwise, through aging, ABCA1 expression and activity declines. ApoE when linked with a lipid conditions its interaction with Aβ, with the prompt transport of the whole complex by the effect of LRP1 and ABCB1 receptor. ABCB1 (ATP-binding cassette sub-family B member 1) is located on the luminal and abluminal side of the endothelial cells' membranes. It is an active mediator for the Aβ peptide transport out from the brain. ApoEε4 protein (lipidated by ABCA1), in relation to the other two alleles, has an evidently weaker linking with Aβ, and the generated complex transport is also weaker. The consequent result is stronger Aβ accumulation in the structures of the perivascular space (PVS), as well as its stronger accumulation in the brain parenchyma. On both levels, its aggregation is stronger. The LRP1 expression drop in the aged has been emphasized previously (***Figs. 4-6***)[25].

Tokuda T *et al.*[26], at the beginning of their study, emphasize that apolipoprotein E (ApoEε4) is the dominant risk factor for sporadic AD (LOAD, 95% of all cases) and familial AD (EOAD, 5% of all cases). ApoE isoforms bind directly with Aβ *in vivo* and *in vitro*. By analyzing this binding, using the lipidated and delipidated ApoE3 and ApoE4 isoforms, Tokuda T *et al.*[26] have found that lipidated ApoE3 isoforms have an evidently higher rate of binding with Aβ than ApoE4 isoforms. This is related to both Aβ1-40 and Aβ1-42. Delipidation of both isoforms leads to the drastic 5-10 fold drop of their affinity towards Aβ, with the loss of difference between them. The injecting of all the isoforms into the special mixture of lipoprotein particles leads to the return of all isoform binding rates to their usual state, especially ApoE3. The strength of isomer binding to Aβ is inversely proportional to the risk for LOAD occurrence. The authors examine the possible use of ApoE3 in the cleaning of the perivascular pathways.

It is important to emphasize the findings by Safieh M *et al.*[27] obtained by analyzing the human CSF, in which is found a markedly lower lipidation of ApoEε4 with HDL, than in the case of ε2 and ε3 isoforms.

The disturbance in Aβ perivascular transport

The passing of Aβ molecules along the BMs which surround VSMCs is characterized by their fairly chaotic movement with mutual approaching and detaching. This is especially expressive in lower velocities and in the higher resistance to the flow. The approach of the two moving neighboring Aβ monomers and their binding is induced by intermolecular hydrophobic interactions, electrostatic interactions, H-bonding, and concurrent cross-linking through advanced glycation endproduct (AGE) compounds from the environment, or formed from precursors in situ, primarily GOLD (glyoxal-lysine dimer) and MOLD (methylglyoxal lysine dimer). In the course of this binding, there is a great possibility for the interaction of MetS35 on one monomer and the MBD (metal binding domain with Fe^{3+}) on the other. The result of this event is a great possibility of an electron "hop" from the S of MetS35 onto the mentioned MBD (Fe^{3+}). This is an ideal situation for the reduction of Fe^{3+} to Fe^{2+}, for the occurrence of the dangerous Fenton reaction, and the generation of aggressive and toxic hydroxyl radicals (*OH).

By means of oxidative attack on the neighboring VSMCs cell membranes and on the protein chains of collagen, *OH steals electrons (e^-), and this leads to serious damage of these structures. This attack with further Aβ aggregation diminishes the strength of vasomotion waves and disturbs the perivascular transport. With the drop of the drainage velocity, conditions are achieved for the failure of arterial walls with changes typical for atherogenesis and the CAA phenomenon[28, 29]. For a better understanding of the mentioned problem, it is necessary to emphasize that the length of Aβ monomer is about 12 nm and the width is about 3.0 nm. The Aβ monomer actually has an amphiphilic nature. Hydrophobic regions lie on the residues 29-40, and the hydrophilic N-terminal domain covers residues 1-28. The diameter of a water molecule is approximately 0.275 nm. The lipidation induced by ABCA1 significantly elevates the hydrophobicity of the central part of HDL and LDL (low-density lipoprotein) and hydrophilicity of their surface. It can be assumed that during the development of the vasomotion wave pressing force, the markedly present hydrophilic complexes on their surface (well soluble in water, *i.e.* in the ICF, with water as a dissolvent), induce an excellent solubility of these particles, with an evidently faster movement along the perivascular drainage pathway. HDL particles diameter, measured by the NMR procedure, in persons with low cardiovascular risk is 9.4-14.0 nm, while in persons with high risk is 7.3-8.2 nm (this is in good correlation with the earlier analysis of ApoEε4/HDL particles). Lamina reticularis of the BMs is otherwise sufficiently wide to enable the drainage of HDL particles, which as well as phospholipids and cholesterol, carry ApoE and Aβ proteins[28, 30].

The driving forces for drainage

VSMC contractions (vasomotion) along the perivascular drainage pathway lead to the intermittent pressure gradient in the arterial media, which drives Aβ molecules dissolved in the ISF and linked with lipids and ApoE protein. The direction of drainage along the BM is contrary to the blood flow in the lumen of arteries. According to the authors of this study, if the surface hydrophilicity of HDL particles, or their polarity, is greater, the resistance to the flow (in fact solubility) is reduced as related to the main element of matrix, *i.e.* water (ECS, ECF). Stronger binding of Aβ with the particles of complex HDL/ApoE by ε2 and ε3 *APOE* alleles, leads to the faster cleaning of Aβ from ECS/ECF

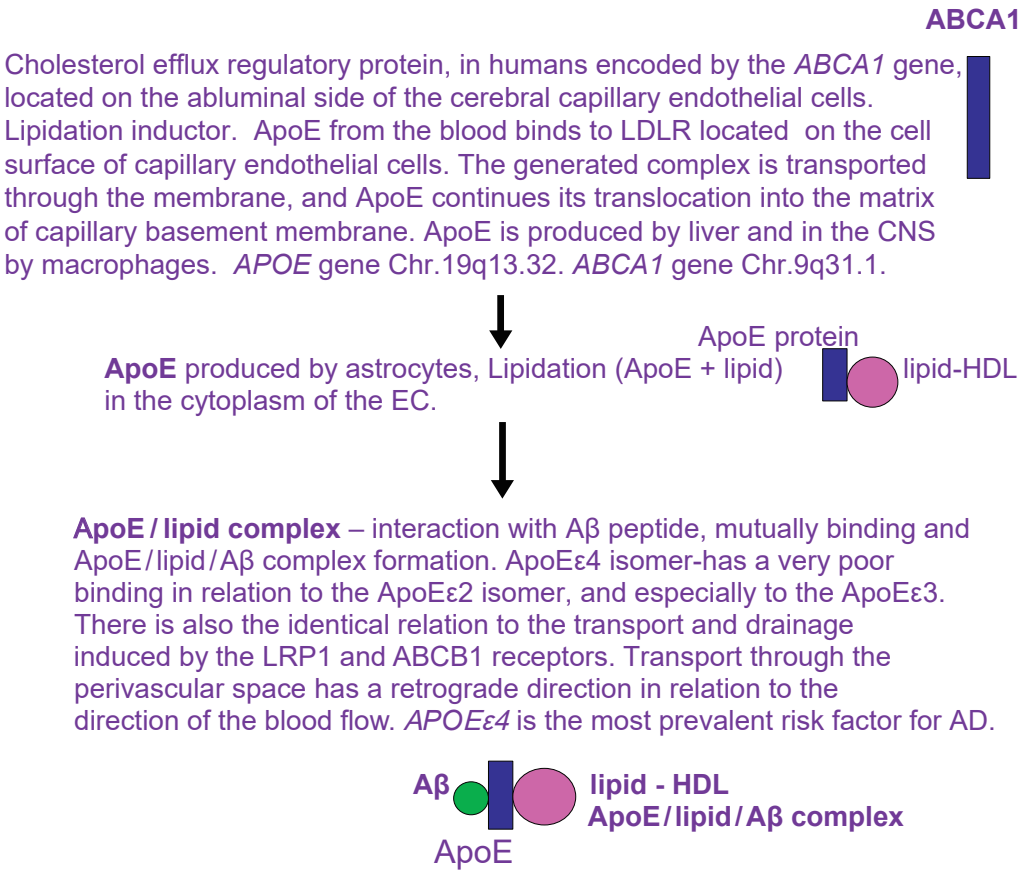

Fig. 6. **Schematic presentation of ApoE protein lipidation and ApoE/lipid/Aβ complex formation.**

The *APOE* gene codes *APOEε2*, *APOEε3* and *APOEε4* alleles. The worldwide frequency of alleles is 8.4% for ε2, 77.9% for ε3, and 13.7% for ε4. In the CNS, microglia and astroglia produce ApoE. ApoE is a transporter for lipids, fat-soluble vitamins and cholesterol. *Chr.19.q13.3* codes all three alleles. It is composed from 299 AAs. ApoE in the brain is the main cholesterol transporter. ApoEε4 is less lipidated by ABCA1 than ApoEε3. Lipidation, the covalent binding of a lipid group to a peptide chain. Astrocyte, star-shaped glial cell of the CNS. Perivascular space, the virtual space located in the capillary basement membranes, and in basement membranes in the media of brain arteries and arterioles. Perivascular space is responsible for drainage of Aβ and waste products out from the brain. LDL, low-density lipoprotein; HDL, high-density lipoprotein; LDLR, LDL receptor, cell surface receptor, composed of 839 AAs, transmembrane glycoprotein, mediates the endocytosis of cholesterol-rich LDL; ABCA1, ATP-binding cassette transporter 1; ABCB1, ATP-binding cassette sub-family B member 1; EC, endothelial cell; ApoE, apolipoprotein E; LRP1, low density lipoprotein receptor-related protein 1. Aβ, amyloid beta; AD, Alzheimer's disease; CNS, central nervous system; AA, amino acid.

(BM interstitium), and its weaker aggregation. ApoEε4 lipidation is weaker (binding with HDL and LDL particles) as related to the other two alleles, which all results in the greater possibility of agglomeration and aggregation of Aβ, in the drop of its drainage, and the development of CAA [4].

The perivascular drainage pathway is actually a tridimensional poroelastic net along which pass collagen and elastic fibers. Lipidated Aβ molecules, located on the Aβ/HDL/ApoE complex, are placed in the way that their hydrophilic parts are congruent with the hydrophilic regions of the other two components. The complete system has an evident hydrophilic surface and is well soluble in ECF, whose major component is water. Lower lipidated Aβ, due to weaker ApoE4 lipidation, and weaker Aβ binding, inclines to its aggregation with the formation of increasingly higher aggregates that are very poorly soluble in water. The restoration of the pressure gradient (vasomotion) leads to the movement of fluid and its present particles. The smaller, lipidated particles can more easily move along the tridimensional net of the pathway, while the greater Aβ aggregates meet a higher resistance, and their movement is significantly retarded. This retardation additionally increases Aβ aggregation. This general retardation of flow raises the local concentration of moving iron ions (Fe^{3+} bound to transferrin). Aβ has a strong affinity towards Fe^{3+}. Aβ aggregation is favorable for the contacts of monomers, electron "hops" from the MetS35 of one monomer to the MBD (Fe^{3+}) of the other, and the Fe^{3+} onset. The generated Fe^{3+} reduction induces the Fenton reaction with the production of the toxic and aggressive *OH. This is followed by further oxidative damages of the local molecular structures (formation of cross-links between neighboring collagen chains-dityrosine and disulfide bridges). The drainage increasingly slows down. For better explanation of these events, it is necessary to present some facts about the iron efflux out from the endothellial cells. FPN1 (ferroportin 1:

transmembrane protein, only known iron exporter) is the iron exporter from cells. But, for this function it is necessary the mutual action of neighbouring protein hephaestin. Hephaestin is a transmembrane copper-dependent ferroxidase, functionally closely bound with the FPN1 receptor, responsible for the oxidation (electron loss) and transport of ferrous iron (Fe^{2+}) from the cell interior across its abluminal cell membrane. The obtained Fe^{3+} binds promptly with the locally present transferrin. Its deficiency results in a serious defect of the mentioned transport [31].

Albargothy NJ *et al.*[3], in their study related to BM, present an excellent pictorial review of the CSF influx pathways in the brain and the drainage of the CSF/ISF mixture along the capillary and periarterial BMs out from the brain. They especially emphasize the entrance of CSF into the brain parenchyma and its mixing in the ECS (extracellular space) with the ISF. The CSF/ISF mixture exits from the ECS and after passing through the narrow paravascular space around capillaries, enters into their BMs, and by vasomotion waves travells to the arteriolar and arterial BMs proximally to the lymph nodes around the carotid. The authors emphasize that the paravascular space is located between the pia mater and astrocytic glia limitans and it is characterized by the presence of α2-laminin. This space practically disappears in the direction towards capillaries. It is important to emphasize that the mixture of fluids from ECS enters the mentioned space through the AQP4 channels and astrocytic endfeet clefts. The authors also point out that the inner and outer BMs of VSMCs do not participate in periarterial drainage. This drainage is exclusively related to the BMs in the media. The authors have not found signs of drainage along the venous walls. Perivascular drainage is extremely important for brain homeostasis, but during the aging process it progressively declines. In the carotid walls, regardless of the subject's age, only a small amount of Aβ molecules have been found.

Weller RO *et al.*[2], present the results of investigations related to the accumulation of insoluble Aβ deposits in the brain parenchyma and cerebral vasculature walls (CCA). In these walls, Aβ accumulation takes place in the arterial and capillary BMs, which are the substrate of perivascular drainage, *i.e.* cerebral lymphatic drainage. According to the authors, the driving forces for this drainage are the arterial pulsations. During the progression of arterial stiffness linked with the aging process, the pulsations decline, resulting in Aβ aggregation in the BMs, a decline in drainage, and the occurrence of CAA. The CAA complications may result in vascular wall ruptures with intracerebral hemorrhages.

The formation of amyloid fibrils

Castillo GM *et al.*[20] investigate the binding of perlecan (specific heparin sulfate proteoglycan) with Aβ(1-40) and Aβ(1-42). The binding capacity closely correlates with the intensity of Aβ fibril formation. Perlecan has also a strong effect on the stability of already formed fibrils.

Bronfman FC *et al.*[17] emphasize that the formation of amyloid fibrils is a nucleation-dependent polymerization process induced by different factors essential for the development, prevention, and treatment of amyloidosis. They present the great capacity of laminin as the inhibitor of Aβ(1-40) fibril formation. They also point out its evident effect to maintain already formed fibrils.

Kiuchi Y *et al.*[18] present data about fibrilogenesis induced by Aβ protein as the cause of AD onset and its progression. According to their investigation, type IV collagen is a strong inhibitor of Aβ fibril formation.

Cotman SL *et al.*[21] emphasize that agrin, extracellular heparan sulfate proteoglycan, according to a number of investigations, has an important role in AD. Its possibility to accelerate Aβ fibril formation as well as to obstruct the Aβ proteolysis has been proven. Agrin is found in senile plaques and cerebrovascular deposits. Cerebral capillaries containing agrin indicate agrin as the cause of pathological alterations in AD.

The role of VSMCs in perivascular drainage

Rannikmäe K *et al.*[32], in their study, investigate the genetic background of CAA. A frequent connection of CAA is established with brain aging, dementia, and lobar intracerebral hemorrhage. A meta-analysis of 24 studies (3,520 examinees) has shown the link of ApoEε4 with CAA. A statistically significant link with ApoEε2 has not been found. However, a specific link is found with the transforming growth factor β1 gene, translocase of outer mitochondrial membrane 40 gene, and complement component receptor gene 1.

Biffi A *et al.*[33], present their findings which show that the carriers of *APOEε2* and *ε4* are exposed to a greater risk of intracerebral hemorrhage (ICH), probably due to the greater risk of CAA.

Aldea R *et al.*[4], in their detailed study, present the experimentally and theoretically confirmed completely new standpoint concerning the role of VSMCs in the generation of the driving force for perivascular drainage. This standpoint is based on the new comprehension that arterial pulsations are not strong enough to be the driving force for the perivascular drainage. The drainage is in fact driven by the innate mechanism of the arterial wall, *i.e.* VSMC contractions and vasomotion phenomenon (V-IPAD, vasomotion driven IPAD). By means of Darcy's hydrodynamic law, the authors have performed a detailed analysis of the fluid flow (here ISF) along the porous reticular lamina of the cerebral arteries media. By this analysis they have obtained a strong confirmation of the VSMCs as generators for the driving force of perivascular drainage (IPAD). The authors do not go into a complex explanation of this hydrodynamic law, adapted for the fluid motion through the brain vasculature. They present components of the essential Darcy's law equation:

$$Q = k*A* (Pb-Pa)/\mu*L.$$

The components of the equation are: Q, total discharge, flow rate (cm^3/sec); A, cross-sectional area to flow (cm^2); k, intrinsic permeability of the medium, coefficient of permeability (cm/sec); Δp, (Pb-Pa), total pressure drop (atm, pascals)); μ, cp, dynamic viscosity; L, length over which the pressure drop is taking place (cm); from the equation it is visible that the Q value is directly proportional to the value of k, A, and Δp, and inversely proportional to μ and L. It should be noted that arterial elongation (L) develops with age. Aβ, by the elevation of its concentration, induces toxic damages in VSMCs and the adequate drop of Pb.

The decline of vasomotion

The perivascular drainage starts with the entrance of Aβ and other waste materials into the structure of capillary BMs, and takes its course along the BMs in the media of cerebral arterioles and arteries.

BMs are poroelastic membranes, in fact special soft plates internally filled with a poroelastic medium, whose pores (diameter ~7-17 μm) open and close depending on the VSMCs relaxation or contraction. The VSMCs contraction, induced by vasomotion waves, forces the fluid filled with dissolved Aβ monomers and other molecules out from the open pores. The fluid flows further in the direction of the vasomotion wave. All these events are completely independent from heartbeat, innervation, and respiration. The vasomotion wave has a length of about 2,000 μm, its period is about 10 sec, average wave speed is 200 μm/sec, and VSMCs contraction strength (S) is about 100 kilopascals (kPa). In the relaxed state, this strength is 0 kPa. This contraction declines during the aging process. The VSMCs contractions drop, induced by increased arterial stiffness and toxic damage caused by the presence of Aβ, generating the decline of the vasomotion induced pressure in the wall (Pb) and the consequent drop of the hydrostatic pressure gradient (Δp, Pb-Pa). The result is the decline of the fluid flow velocity and the increased tendency for stronger Aβ aggregation (***Fig. 5***)[4, 13, 19].

As previously mentioned, vasomotion is a vascular phenomenon, defined as spontaneous oscillations of the vascular musculature tension, independent from heartbeat, pulse wave moving, innervation, and respiration. Their origin lies in the rhythmic intracellular oscillations of Ca^{2+} ions concentration in the VSMCs. The starting point of the vasomotion wave is related to small arterioles, metarterioles, and precapillary sphincters. According to Raffaello A *et al.*[8], the mentioned Ca^{2+} concentration oscillations are the result of its periodical release from stores located in the VSMCs endoplasmic reticulum (ER). Gaspers LD *et al.*[9], in their study also present the complete mechanisms underlying the vasomotion phenomenon. Baric N[6], in his last paper, has also presented a comprehensive review related to this problem.

Kalaria RN *et al.*[34] present the changes in cerebral capillaries connected with aging. During the process of aging, in the cerebral capillary endothelium (BBB) the loss of cytoplasm, drop of mitochondria number, loss of strong connections between endothelial cells (tight junctions) and the thickening of the BMs linked with collagen accumulation occur. Astrocytic endfeet retract and the alteration of pericytes occurs. Linked with these changes, there are often lacunar infarcts, cortical and subcortical microinfarcts, microhemorrhages and diffuse damage of white matter linked with the loss of myelin and alterations in axon structure.

Thomsen MS *et al.*[35] emphasize the importance of the BMs which contribute to the integrity of BBB composed from the capillary endothelial cells. The BMs width is about 20-200 nm. In fact they are a tridimensional network, mainly formed with proteins which belong to the big glycoprotein groups: laminins, collagen IV isoforms, nidogens, and HSPGs. Apart from them, in these groups there are also some other proteins: insoluble fibronectin, fibulin 1 and 2, collagen type XVIII, thrombospondins 1 and SPARC (secreted protein acidic and rich in cysteine). The present integrins and dystroglycan contribute to a better interrelated connection of the mentioned proteins. The changes in the structure and composition of BMs in AD include the sedimentation of Aβ, their thickening, and changes in protein structure. Laminins, collagen IV, and nidogen, break the Aβ1-40 and Aβ1-42 fibril formation. In AD, the concentration of perlecan and fibronectin in capillaries is elevated.

The lymphatic drainage pathways

Bakker ENT *et al.*[36] emphasize that the lymphatic drainage pathways in the brain are completely different as compared to the other parts of the body. They are not only important for the regulation of ISF and CSF volume, but also for the drainage of waste products from the brain, among them especially Aβ. The cleaning of this waste predominantly takes place while sleeping. The authors emphasize the established communication between ISF and CSF, as well as the connection between CAA and disorders of the perivascular drainage. Astrocytic endfeet form a complete circle around capillaries. Between astrocytic endfeet and capillary endothelium there is a space 40-100 nm wide filled with the capillary BM. In the capillary region, this membrane is in fact composed of two joined layers, outer and inner BM of VSMCs. Paravascular space on the capillary level is very narrow and it is located between the astrocytic endfeet and the outer surface of the joint membranes. Between the BM and ECS there is good communication by AQP4 channels and intercellular astrocytic endfeet clefts (***Figs. 3-5***). In this region, the pia is practically absent. Contractile pericytes are attached close to the endfeet. The width of the basal lamina (bl.) is on an average about 100 nm. It is produced by endothelial cells and VSMCs. After entering into the BM, ISF drains further on by bulk flow along its interior. According to the authors, the BMs are dominantly composed of collagen type IV, laminin, fibronectin and heparan sulfate proteoglycans. They possess the capability of autoremodeling. Confocal microscopy has shown that tracers co-localize with laminin in the capillary BMs and arterial tunica media.

Marques F *et al.*[37] present a detailed analysis of aging alterations in the cellular elements of neurovascular units, and in the choroidal plexus (CP) endothelium. They emphasize the endothelial necrosis, accumulation of the extracellular matrix components in the VSMCs, the drop of mitochondria in the cells, the occurrence of pinocytotic vesicles, loss of tight junctions, retreat of astrocytic endfeet, stiffening of vessel walls and loss of elasticity. Regional blood flow and metabolism decline, as well as the oxidative processes, and the whole drainage process.

Prevention and therapy for AD

After this presentation, it is clear that presently an effective prevention and therapy for AD and CAA still does not exist. The cleaning of the perivascular drainage pathway will also be a great problem in the future. The actual therapy for AD with cholinesterase blockers (donepezil, rivastigmine, galantamine) is not satisfactory, and a number of other current therapeutics have a problematic effect. Currently, there are attempts with immunotherapy, therapy with free radical scavengers and chelators (for example curcumin). Cross-

linker breakers is also widely used in therapy. The correction of fasting glucose level (nonenzymatic glycosylation of Aβ retardation) has an important preventive role. Generally speaking, AD therapy presently includes antioxidants, AGE breakers, RAGE (Receptor for AGEs) blockers and anti-glycation compounds.

Recently, researchers are directed to the effects of alagebrium (ALT-711), aminoguanidine, DPTC, tiamine, benfotiamine, and piridoxamine. Related to the extracellular degradation of Aβ, the effects of neprilysin (metalloendopeptidase) and matrix-metalloproteinases 2, 3 and 9 inhibitors, are intensively investigated, as well as functions of proteasomes and the ubiquitin-proteasome route. It is also necessary to mention vitamin C, vitamin E, superoxide dismutase (SOD), Ginkgo biloba, and green tea (among the polyphenols found in green tea the most important is epigallocatechin-EGCG)[5,6,38-41].

It is evident that during the aging process, especially with the presence of AD, there is a breakdown of many cerebral functions. The recognition and comprehension of this breakdown, as well as its prevention and therapy, all require an intensive approach; otherwise we can expect serious disturbances of social relations in human society with unforeseeable consequences.

Conclusion

Due to the increasing rise of AD incidence and prevalence, this serious, chronic, and at the end lethal disease, is becoming one of the crucial problems of modern society. The world population is increasingly older, absolutely and relatively. AD is closely connected with age and aging. This neurodegenerative disease with a chronic and progressive course, is marked with a progressive decline of memory, disorientation, and general drop of cognitive functions. The costs for its prevention and therapy exponentially grow. Due to the great importance of Aβ accumulation and aggregation in the AD brain, it is evident and logical that a number of recent investigators and their institutions increasingly take part in the research of the etiology, genetics, and pathophysiology of this disease. These investigations are especially related to the failures of perivascular drainage and modes of its improvement. The presented study is obviously also included in this research.

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Conflict of interest

The authors claim no conflict of interest in this study.

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