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Original article

Increase in iron intracerebral concentration in patients suffering from Alzheimer's disease follows the rise of amyloid beta.

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Abstract

***Objective*:** The process of iron and amyloid beta (Aβ) intracerebral accumulation represents the focus of Alzheimer's disease (AD) pathophysiology. This disease with its yet unexplained ethiology is chronic, wasting, progressive and lethal. There exists a clear connection with the aging process and old age. The mentioned accumulation of iron and Aβ is dependent on the disturbed balance between the iron influx and drainage, as well as the Aβ production, destruction and drainage. In AD evident is the elevated Aβ production, decline in its destruction, and increase in its accumulation in important routes of drainage. These routes are in fact the same as for iron: the iron elimination from the brain declines, and its accumulation in the brain interstitium elevates. This elevation leads to the rise of the dangerous Fenton reaction and enormous production of the aggresive and toxic hydroxyl radical (*OH). The forming of the strong continuous oxido/reductive cycles leads to a permanent production of deteriorative reactive oxygen species and AD pathophysiology.

***Methods*:** By means of a detailed insight into a number of relevant studies about the role of iron ions and Aβ in AD pathophysiology, and after consulting some respected investigators in this area, the author presents a review of the mentioned events.

***Results*:** The results of the undertaken analysis indicate the great importance of metal ions, especially iron ions, and Aβ peptide in the origin and course of AD. The results especially emphasize the importance of drainage routes for both subjects and their blockade.

***Discussion*:** This study presents the results of investigations undertaken by a number of prominent experts. They all suport the idea of the great importance of iron and Aβ accumulation in the ethiology of AD. They also emphasize the role of their altered drainage routes.

***Conclusion*:** AD is a wasting, chronic and lethal neurodegenerative disease. The explanation of its polygenetic ethiology is still unsatisfactory. The investigation of Aβ and iron drainage routes should be one of the crucial imperatives in the understanding of this disease.

***KEY WORDS*:** Alzheimer's disease, amyloid beta, iron ions, oxidative stress, drainage routes.

Introduction

The aim of this study is to give some crucial facts about the fundaments of Alzheimer's disease (AD) pathology and pathophysiology, without entering a detailed presentation of the clinical picture and course of this terrible lethal disease.

Following the expressive rise of worldwide old population, there is a concomitant occurrence in the incidence and prevalence of chronic degenerative diseases, among them especially neurodegenerative, with a marked position of Alzheimer's disease AD. AD is a chronic, progressive and lethal disease, still mostly of unknown polygenetic etiology. It is closely connected to the aging process and old age. Due to its clinical picture with typical progressive memory loss, as well as the loss of thought and speech ability, this terrible and at the end lethal disease enormously burdens the patient's family, as well as the complete society.The nowadays costs for its prevention and treatment unfortunately have an exponential trend [1-4].

Before further analysis, it is necessary to emphasize the existence of its two essential forms: the early form (EOAD, early onset AD, 5% of all cases) which develops before the age of 65, and the late form (LOAD, late onset AD, 95% of

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all cases) which develops after the age of 65. The clinical picture of both forms is practically the same [1,2-4].

The complex of known mutations: APP (β-amyloid precursor protein gene, 21q21.3); PSEN1 (presenilin-1 protein coding gene, 14q24.2); PSEN2 (presenilin-2 protein coding gene, 1q42.13), and BACE1 (β secretase-1 protein coding gene, 11q23.3) lead to EOAD, and APOEε4 allele of APOE gene (apolipoprotein E coding gene, 19q13.32), ADAM10 (ADAM metallopeptidase Domain 10 protein coding gene-regulator of α-secretase activity-gene, 15q21.3), and TREM2 gene (6p21.1 – encoding the triggering receptor protein expressed on myeloid cells 2), lead to LOAD. It is also necessary to mention a set of other genes connected with the onset and course of LOAD. They are: ABCA7, CLU, CR1, PICALM, PLD3, and SORL1. Their activity is presently under intensive investigations [1,2-4].

According to a number of recent investigations about the AD patophysiology, strong facts indicate the crucial role of oxidative stress. This type of stress, different from glycative stress, primarily is induced by metal dyshomeostasis, especially with the intracerebral rise of transition metals, among them on the first place iron (Fe) and copper (Cu). These two metals have great affinity toward the amyloid beta (Aβ), an important protein whose concentration, like in the mentioned metals, is in AD brain markedly elevated. These metals, especially Fe^{3+} (ferric iron), can be by Aβ reduced (electron gain) in their redox active toxic form Fe^{2+} (ferrous iron), which can enter the Fenton reaction, and generate the extremely toxic, aggressive and destructive hydroxyl radical *OH. The result is a strong oxidative deterioration of many vital molecular structures like lipid membranes, proteins, and DNA molecules. For the strong intracerebral Aβ increase there exists a recently well documented genetic explanation. But, for the intracerebral increase of iron (Fe^{2+} and Fe^{3+}) there are yet many unexplained questions [1,2-4,5].

Without entering the detailed explanation of iron uptake from food, its travelling through the gastrointestinal tract and consequent absorbtion in the blood, it is necessary to present some facts about its passing across the blood brain barrier (BBB) and its entrance into the brain intercellular space (neuropil). Conjugated with the protein transferin (Tf – generated in the liver) two ferric ions (Fe^{3+}) become accepted by the BBB capillary membrane transferin receptor (TfR), included in the structure of the endosome, and in its cavity reduced by duodenal cytochrome b to ferrous iron Fe^{2+}. Transported by the divalent metal transporter 1 (DMT1) located in the endosome wall, reduced Fe^{2+} enters the capillary stroma, and then by the action of ferroportin1 (FPN1) every unit of Fe^{2+} is exported into the local extracellular fluid (neuropil). After a quick contact with the locally present astrocyte β-amyloid precursor protein (APP) and ceruloplasmin (CP), Fe^{2+} becomes oxidised into Fe^{3+} and also by the locally present Tf generated by oligodendrocytes, it enters with the cooperation of the neuronal membrane TfR into the neuronal stroma. In the neuronal endosome, Fe^{3+} is, by ferric reductase, reduced to Fe^{2+} and, by DMT1 and FPN1, exits out from the neuron into the neuropil. Then it can be oxidised by the above mentioned APP and CP into Fe^{3+}. Fe^{3+} can be reduced by the local Aβ into Fe^{2+} and can enter the Fenton reaction with the generation of the strong destructive *OH (***Fig. 1***) [6-8].

Before further analysis, it is necessary to present a number of important iron functions connected with the brain. They are: oxygen transport by blood across the brain arteries, participation in the energy production in the brain cells (ATP production), myelin production by oligodendrocytes, maintaining the immune system, the important function in the activity of the electron transport chain (mitochondria), and production of some brain neurotransmiters. The intracranial concentration of iron, important for optimal functioning of brain cells, must be in constant balance, and any deviation from this balance is unfavorable for brain cells. There are several regulatory systems to prevent the disbalance. In the case of elevated iron concentrations, iron, by means of some receptors (especially TfR) and transporters (Tf has a crucial role), in the form of Fe^{3+} and Fe^{2+} enters the nerve cells and after the Fe^{2+} oxidation, stores in the form of Fe^{3+} (redox-inactive, non toxic form) in the intracellular protein apoferritin, forming ferritin. In the case of iron concentration drop, ferritin, after the Fe^{3+} reduction, releases Fe^{2+} ions in the interstitium and elevates its concentration. Every failure of iron storing and releasing from cellular ferritin has an unfavorable effect on the brain cells [6].

What are the causes of the high intracerebral iron concentrations?

It is evident that the influx of iron through the BBB is constant. It is also clear that there has to be an effective exit for the elevated iron ions. Considering these facts, some further explanation is necessary. Fe^{3+} concentration is equal in the extracellular space (ECS) and in the cerebrospinal fluid (CSF) of subarachnoid space (SAS). There is no blockade in the transit between these two systems (pia is very permeable). Iron ions in the SAS (CSF) enter into the arachnoid villi (AV - several villi make the arachnoid granulations [AG]; AV are the integral components of AG), and conjugated with the Tf (generated by oligodendrocytes), they exit into the venous blood of the superior sagital sinus. The understanding of this process and its alteration requires detailed knowledge about the AV and the granulation (AG) structure. AG, are essentially composed of the pedicle, body, and apex, and are surrounded by the connective tissue capsule. This capsule is composed of collagen bundles which on the surface encircle small holes with different shapes and magnitude. As is earlier emphasized, AG are composed of the more integrated villi. Small holes are limited by thin filaments and the greater ones by thicker filaments. The AG interior is built from the fibrous tridimensional net with interpolated circular cavities. Some AG, visible only by eye, are usually composed of several smaller units which are visible only by microsope (AV). Connective AG fibers generate from the dura. AG are primarily located in the superior sagital sinus near the venous openings. A light microscopic study of a typical AG shows the central core built of the slack connective tissue, and the peripheral region formed from dense connective tissue projecting through the dura of the sinus wall. At the AG base, visible is the adjacent venous structure. On the surface of the AG, the endothelium is visible, which is in fact the prolongation of the sinus endothelial covering. As to the CSF transport, and the CSF absorption, some authors consider that there is a passive transport through the direct canal system. Other authors emphasize the active transport, and some include both mechanisms. According to all these opinions it is most probable that the Aβ, Tf-Fe^{3+}, and other waste materials in the AG, are included in endothelial cells, as well as in the

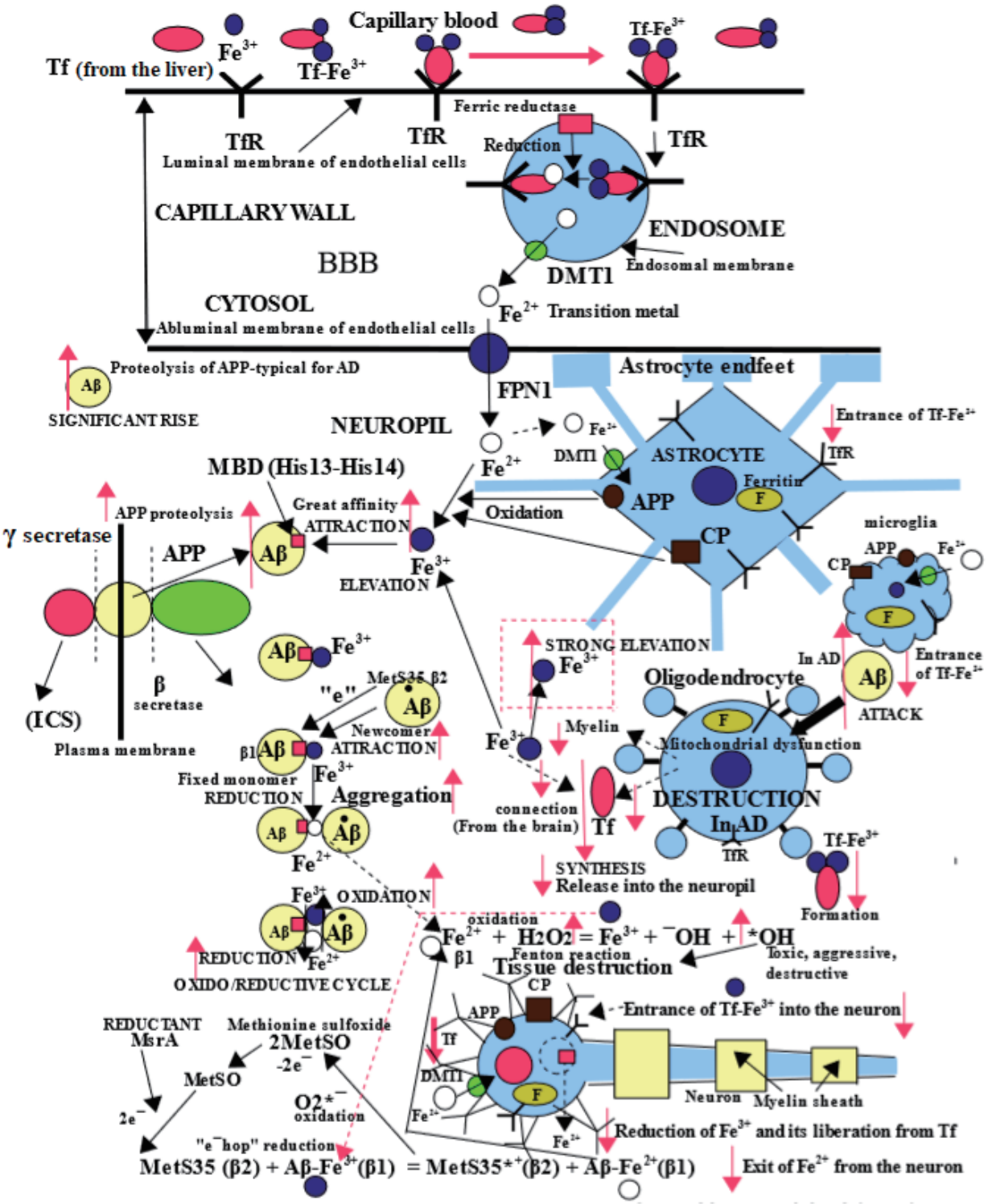

Fig. 1. **Schematic presentation of events connected with Fe^{3+} elevated intracranial values.**

A small part of Fe^{2+} enters the cells by DMT1 and stores in ferritin.

AD, Alzheimer's disease; Aβ, amyloid beta; APP, amyloid β precursor protein; CP, ceruloplasmin; BBB, blood brain barrier; ICS, intra cellular space; ECS, extra cellular space; MBD, metal binding domain; Tf, transferrin; TfR, transferrin receptor; DMT1, divalent, metal transporter 1; FPN1, ferroportin 1; F, ferritin; ■, ferric reductase; Met, methionine; MetS, MetS35, methionine sulfide; $MetS35^{*+}$, methionine sulfide radical.

layers of arachnoid cells. Endothelial cells are abundant with micropinocytotic vesicles and intracytoplasmic vacuoles. The arachnoid cell layer contains a number of extracellular cisterns. All these facts suggest that in reality a direct canal net does not exist. Generally, the preference is on the active transport. If the cavity of the granulations is filled with the deposited Aβ (elevated in AD), the iron ions efflux is markedly decreased (***Fig. 2***) [8-10].

The elevated intracerebral interstitial iron concentration causes typical oligodendrocyte damage, which has been proved by a number of examinations. This damage is connected to elevated Aβ concentration (typical for AD), which has an unfavorable effect on the oligodendrocyte structure and function. The scenario of these events is probably as follows: By FPN1 effect, Fe^{2+} is exported from the capillary wall into the extracellular space where it is promptly oxidised by APP and CP, which are both located on the plasma membrane of neurons, microglia and especially astrocytes (astrocytes are located close to the capillary wall). With significant alterations of myelin production induced by oligodendrocytes, Aβ also provokes in these cells a decreased production and secretion of Tf, very important for Fe^{3+} transport. Tf, together with TfR, transports Tf-Fe^{3+} into the neuron stroma and the present endosome. When the Tf production declines, Fe^{3+} enters very poorly into the neurons. In this case, there is no reduction in the endosome, and Fe^{2+} is poorly generated. Consequently, a deffect occurs in the FPN1 induced exit of Fe^{2+} into the interstitium. Aβ has otherwise a marked affinity for Fe^{3+} and binds it to its metal binding domain (MBD - His13/His14) on the fixed Aβ monomer (the apex of the forming protofilament). Monomer aggregation, at the beginning still nonparallel and noncongruent, leads to the close encounter between MBD of the fixed monomer and MetS35 of the incoming monomer. The close encounter (critical distance is below 19Å) leads to the electron "hop" from MetS35 S (the incoming monomer) onto the MBD Fe^{3+} (the fixed monomer) and to the accompanying Fe^{3+} reduction (electron gain). The developed Fe^{2+} enters into Fenton reaction with the consequent generation of the markedly toxic and aggressive hydroxyl radical *OH ($Fe^{2+} + H_2O_2 = Fe^{3+} + {}^{-}OH + {}^{*}OH$). In this manner, the evident small amount of Fe^{2+} (non-Tf-bound iron, NTBI) enters the cells (neurons, astrocytes, microglia), because Fe^{2+} is captured by APP and CP with the oxidative transformation into Fe^{3+}. Due to the Fe^{2+} diminished concentration, its storing into ferritin is also reduced. So Fe^{2+} is much more included into Fenton reaction, with the Fe^{3+} and *OH generation. Through time, the reaction becomes stronger. Oxido/reductive events in the form of Fenton reaction, and Fe^{3+} reduction induced by Aβ, both collect all the arrived Fe^{3+} which are obtained by the APP and CP oxidised Fe^{2+} into Fe^{3+}. All this is accompanied with the growing intensity of these reactions. In this manner, the normal iron storing into the cells and into ferritin is disturbed. In the ECS, the Fe^{3+} concentration significantly rises. The exit from the actual situation should be an intensive Fe^{3+} Aβ drainage, but, in contrast to this, evident unfavourable events appear. A small amount of Fe^{2+} which is not oxidised (CP, APP), by the DMT1 enters into the cells (astrocytes, neurons, microglia), and after the oxidation in form of Fe^{3+} stores into the ferritin. Due to the elevated values of Fe^{3+} interstitial concentration, there are no signals for releasing Fe^{2+} from ferritin stores (***Fig. 1***) [5, 6, 11, 12].

This study in detail elaborates a number of data about the problem of Fe^{3+} efflux from the intercellular space (ICS) in the AD brain, based on recent literature involved in the same problem.

Results of available analysed studies on AD pathophysiology indicate the crucial role of oxidative stress induced by metals. Especially emphasized is the role of the iron ions efflux and its defects. The orderly efflux has a great importance in maintaining the optimal intracerebral iron concentration, in the prevention of oxidative stress with tissue destruction, and in the influence on the course of AD. The role of reactive oxygen species (ROS), especially *OH is also emphasized [1, 2-5, 6].

Discussion

Iron transit from the blood to the neuropil

By experiments in healthy subjects, a disproportion is found between the intracranially detected lower elevation of the iron ions concentration depending upon the age on the one hand, and on the other, the expected greater elevation based on the assessment of the rate of the iron transit from the blood through the BBB into the neuropil, also depending on the age. Adequate rates were determined on the basis of exact investigations and measurements. The obtained disproportion (healthy people) suggests a constant drainage of specific amount of iron from the neuropil [7, 8]. Experiments conducted on rats by punctions and by carrying radiolabeled Tf into the lateral cerebral cavity, indicate that this compound is mainly quickly reabsorbed into the blood stream, and only a small amount is used for the necessity of cerebral cells. These experiments show that transferin (Tf-Fe^{3+}), carrying iron, is removed from the interstitial (ISF) and cerebrospinal (CSF) fluid, mostly probably into the AG, and from these through the arachnoid membrane and endothelium of the great venous sinuses it enters the venous blood stream. It is evident that in this case, Tf has a reversed direction towards to the venous sinus blood. In the CSF, Tf is completely saturated with iron (Tf-Fe^{3+}), however, its concentration is here very low. The authors of these investigations consider that due to these facts, the iron transport by this route is insignificant. In the period of these experiments, other posible routes of the ISF efflux were still unexplored, so the crucial role for this efflux was exclusively attributed to the AG and their components (AV) [7, 8].

The role of CSF

Silverberg GD *et al.* [13] emphasize that the production and turnover of CSF are important for the cleaning of toxic molecules, especially Aβ, from the ICS, and for their transfer to the blood stream. During the process of ageing, a lower CSF production develops as well as a great resistance to CSF outflow. In the circumstances of the CSF reduced production related to the evident elevation of CSF outflow resistance, occurs the great possibility of AD development. The elevated CSF outflow resistance in AD is evidently the consequence of the increased Aβ deposition in the meninges.

In another study, Silverberg GD *et al.* [14] again emphasize the great possibiliy of AD in the predomination of reduced CSF production and impaired choroid plexus transport.

Tarasoff-Conway JM *et al.* [15] present a comprehensive study about the clearance systems in the brain with implications for AD. They emphasize that both AD types

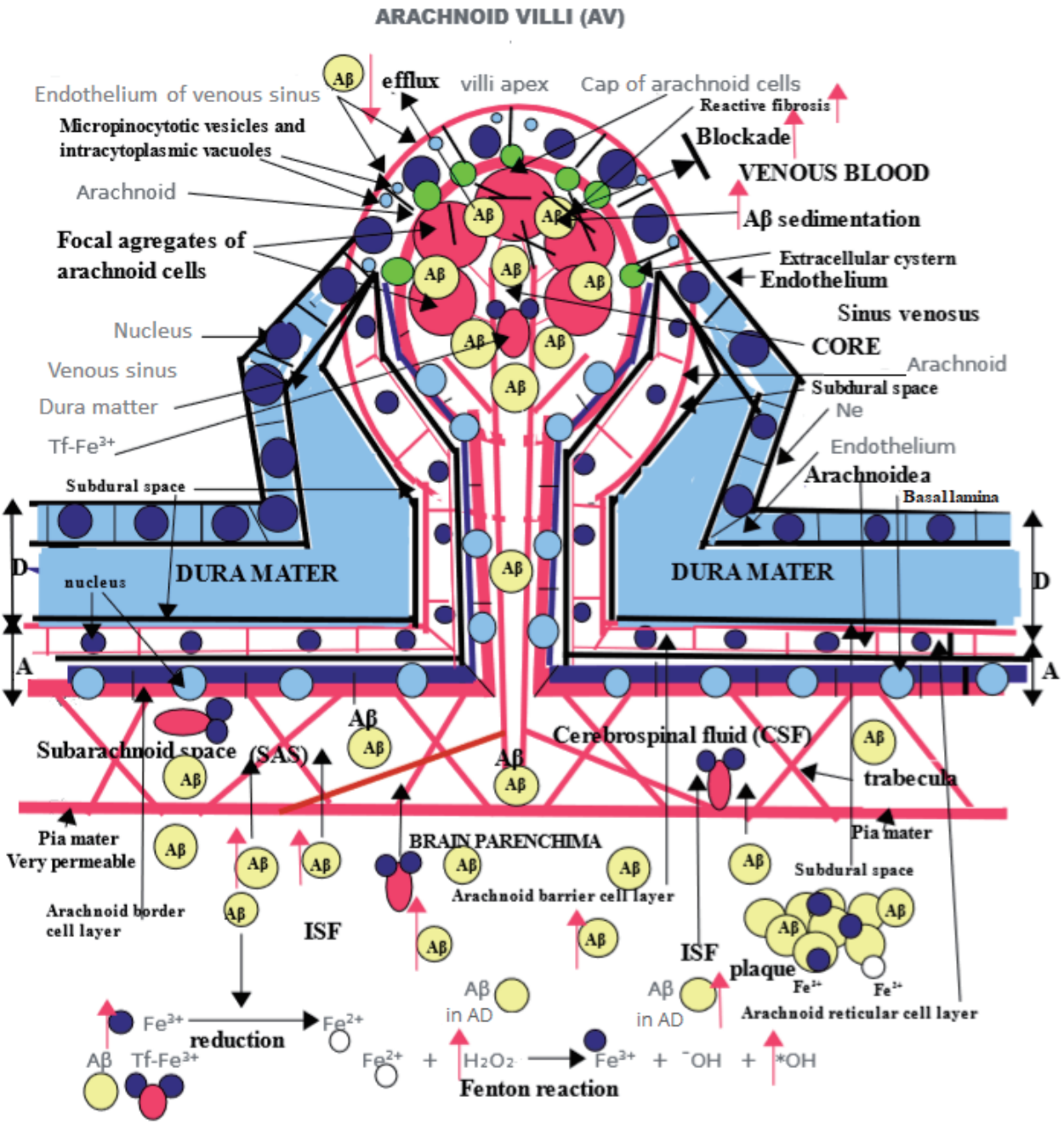

Fig. 2. **Schematic presentation of the arachnoid villi (AV) and Aβ sedimentation.**

AG are visible to the unaided eye and AV only by microscope. The elevated Aβ influx in the AG space is the reason for its enormous local sedimentation and consequent failure in efflux in the venous sinus blood. The Aβ sedimentation induces decreased cerebrospinal bulk outflow (CSF) and increased outflow resistance at the AV (arachnoid villi; the microscopic parts of AG).

AD, Alzheimer's disease; Aβ, amyloid beta; AV, arachnoid villi; AG, arachnoid granulations; Tf, transferrin; Fe^{3+}, ferric iron, redox-inactive nontoxic ion; CSF, cerebrospinal fluid; SAS, subarachnoid space; ECS, extra-cellular space; ISF, interstitial fluid; *OH, hydroxyl radical; NE, nerve ending.

originate due to strong intracranial Aβ accumulation. This accumulation in fact originates as a result of the disordered balance between the Aβ production and its cleaning. In EOAD there exists the elevated production and decreased cleaning, and in LOAD exists only decreased cleaning. The authors emphasize a number of recently known cleaning systems of disolved Aβ: Enzymatic degradation and cellular uptake, transport accross the BBB (specialized transporters), blood-cerebrospinal fluid barrier (BCSFB), ISF bulk flow facilitated by astroglyal aquaporin-4 (AQP4) channels, CSF absorption into the circulatory and lymphatic system, and meningeal lymphatic vessels (discovered 2015). The exact proportion of every of these systems in the Aβ cleaning is not yet exactly determined. However, the damage of any of these systems contribute to typical damages of AD. This study shows very interesting data about the role of perivascular and paravascular drainage of waste products, Aβ, and iron out from the brain. Moreover, it also in detail elaborates the special paravascular space (the space located in the pial funel extending between the vessel pia and cortical pia, the so called Virchow-Robin space [VRS]). Alterations of the perivascular and paravascular systems have an important, actually a crucial role in the pathology and patophysyology of AD. In the subsequent presentation, these systems will be again analysed. The above mentioned authors also especially emphasize the Aβ accumulation in the cerebral arteries and capillary walls (cerebral amyloid angiopathy; CAA). Otherwise, Aβ cleaning through BBB, according to a number of investigations, has a dominant role.

Weller RO *et al.* [16] emphasize that the perivascular ISF drainage of dissolved metabolites and Aβ originates in the capillary region where these compounds enter the capillary basement membranes region limited by the endothelial cells and surrounding astrocytes. Their route further follows the entrance into the region between the inner and outer basement membranes of arterioles and arteries between the vascular smooth muscle cells (tunica media). The direction of waste products and Aβ through the arterial wall has an inverse direction in relation to the direction of the blood. The flow of the mentioned compounds through the arterial wall leads to the cervical lymph nodes located along the carotid arteries (***Fig. 3-4***).

The tight drainage route separated from the surface of the penetrating artery by mentioned VRS, transports CSF, ISF, waste products and Aβ in the direction parallelly to the blood flow (paravascular space). VRS is limited on the interior side (towards the artery) by the vessel pia, closely attached to the outer basement membrane of smooth muscle cells, and on the outer side it is limited by cortical pia and astrocytes *i.e.* the basement membrane of glia limitans (paravascular drainage route). The role of this space (VRS) in the possible drainage is presently under intensive investigations. The compounds drained by paravascular drainage rout exit into the region of the capillary/venule contact, and continue further on the paravascular route close to the vein surface in the direction to the venous sinuses (***Fig. 3-4***) [15, 16].

Aβ drainage

Moris AW *et al.* [17] present a detailed description of the suppression of Aβ elimination through perivascular drainage routes, and they consider this suppression as an important factor in the AD developement. This suppressed elimination is in fact the consequence of Aβ sedimentation in the range of cerebral amyloid angiopathy. They are especially occupied with the role of cerebral vascular basement membranes as pathways for the passage of fluid out from the brain. These routes in fact correspond to the conventional lymphatics in other parts of the body. The description is practically the same as the findings of Weller RO *et al.* [16].

Sakka L *et al.* [18], in their study, give the description of the elementary characteristics related to CSF. The mean value of CSF volume is about 150 mL, 25 mL in ventricles and 125 mL in SAS. CSF is primarily generated in the choroid plexus. Among a number of factors, pulse waves have a primary effect on the CSF circulation. Parallel to the effects of cranial and spinal arachnoid villi (*i.e.* AG), the cranial and spinal nerve sheats, cribriform plate, and adventitia of cerebral arteries also have effects on the CSF reabsorption into the venous outflow system. In the course of 24 hours, the CSF is completely restored. During the process of ageing, the originated CSF turnower reduction leads to the catabolites accumulation in the brain, which in any case contribute to the occurrence of neurodegenerative diseases.

Morison BM [19] gives some knowledge about arachnoid villi and arachnoid granulations. Different from microscopic AV, AG are macroscopic structures composed of a large number of villis. AV in fact are unidirectional ventiles responsible for the CSF absorption into the venous blood. A minimal pressure of 20 mm H_2O is necessary for the absorption through AV. According to this author, the absorption through AV is obtained by bulk flow.

All the above presented facts indicate that in AD there is a strong rise in the resistance of CSF outflow through arachnoid granulations (CSF outflow resistance) into the large venous sinuses. The explanation probably lies in the strong Aβ sedimentation in the mentioned granulations typical for AD, and in the probably accompanying reactive fibrosis. It is clear that this event, according to the decreased outflow of CSF, contributes to the rise in iron ions concentration in the neuropil [13, 14].

The explanation of the strong intracranial rise of the iron ions in AD requires a detailed knowledge about the routes of its drainage, because it is obvious that their blockade or damages lead to the mentioned rise of iron ions and to a greater possibility of fatal effects of oxidative stress. Along with the mentioned drainage through AG, some other routes are presented further on in this study. It is also necessary to mention the process of iron storage by ferritin.

The CSF drainage through the periarterial space of the penetrating artery flows in the opposite direction as to the direction of the blood. This drainage originates in the capillary system, and ends in the cervical lymph nodes. CSF also enters into the paravascular drainage route and continues through this route, circulatory limited by pia and the basement membrane of glia limitans, parallelly to the blood flow direction up to the capillary system. It is observed that during this flow, a part of CSF passes through the outer border line (basement membrane of glia limitans) and returns (across AQP4 cannals) into the ECS, mixing there with the present ISF (***Fig. 3-4***) [15, 20].

The next route of CSF drainage is connected with the perineural space around the cerebral nerves.This route is in fact the SAS extension and it is clear that it is limited with

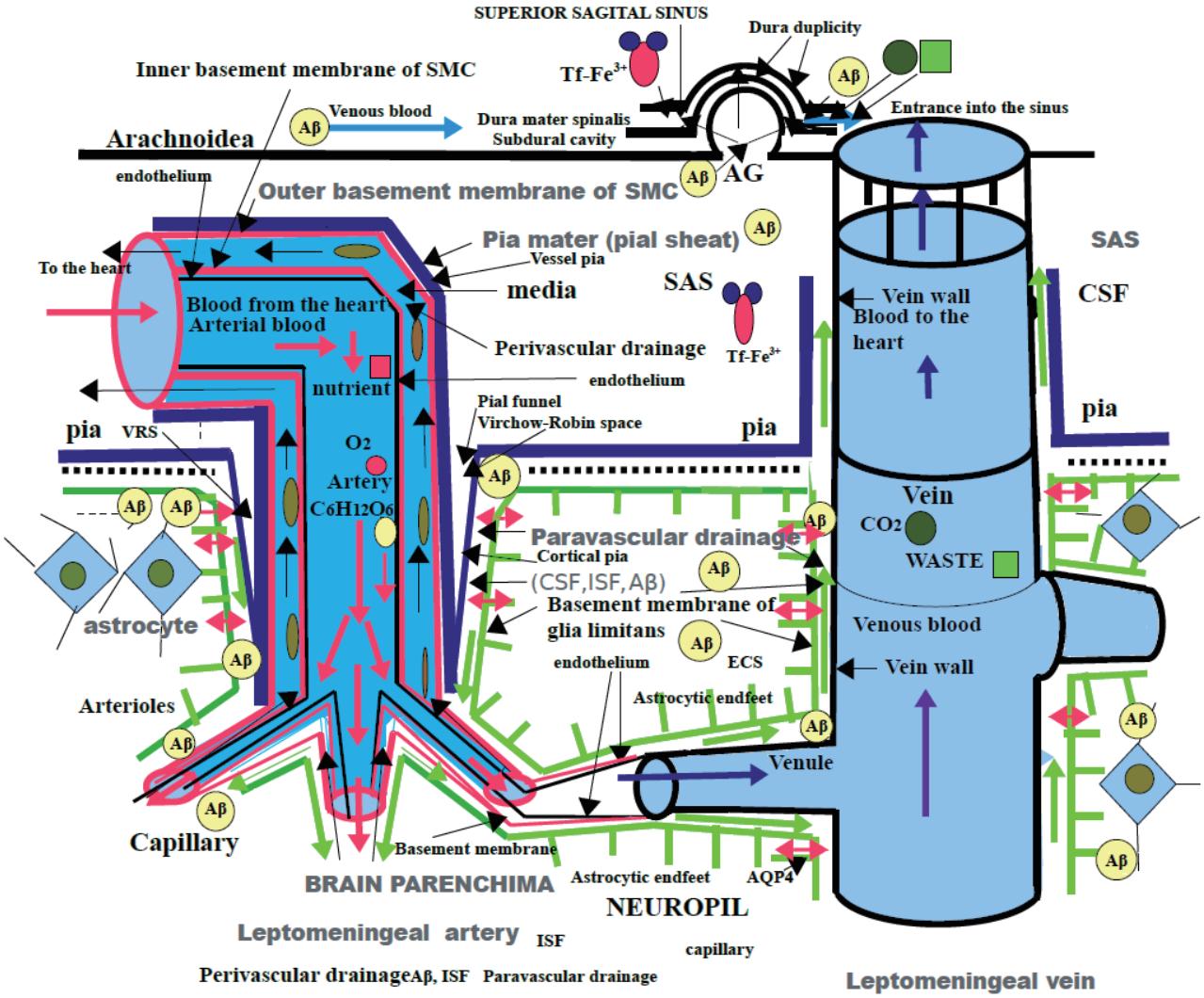

Fig. 3. **Schematic presentation of the Aβ, iron, and waste products drainage from the brain cortex.**

Arterial blood transports oxygen and nutrients; venous blood transports carbon dioxide and metabolic waste products; along with these two fundamental routes, the figure presents also two important pathways, the perivascular and paravascular drainage systems; both systems are clearly visible on the left side of the figure-leptomeningeal artery. Astrocytes endfeet form the outer basement membrane of glia limitans; this membrane is the outer wall of paravascular drainage system; by this system CSF, ISF, Aβ, iron ions, and waste are transported along the artery wall parallel with the direction of blood flow. Pial sheat closely connected with the outer membrane of SMCs presents the internal wall; this pathway along the penetrating artery lies in the space called paravascular drainage space. Between the outer and inner basement membranes, through the media, there is a perivascular drainage pathway which transports ISF and Aβ contrary to the direction of the blood flow, in the direction towards the heart; the improvement of Aβ drainage along the perivascular pathway should be one of the crucial tasks in future investigations.

Aβ, amyloid beta; AV, arachnoid villi; AG, arachnoid granulations; CSF, cerebrospinal fluid; SAS, subarachnoid space; Tf, transferrin; Fe^{3+}, ferric iron; VRS, Virchow-Robin space; AQP4, aquaporin-4, channels located on astrocytic endfeet, important for Aβ drainage; SMCs, smooth muscle cells.

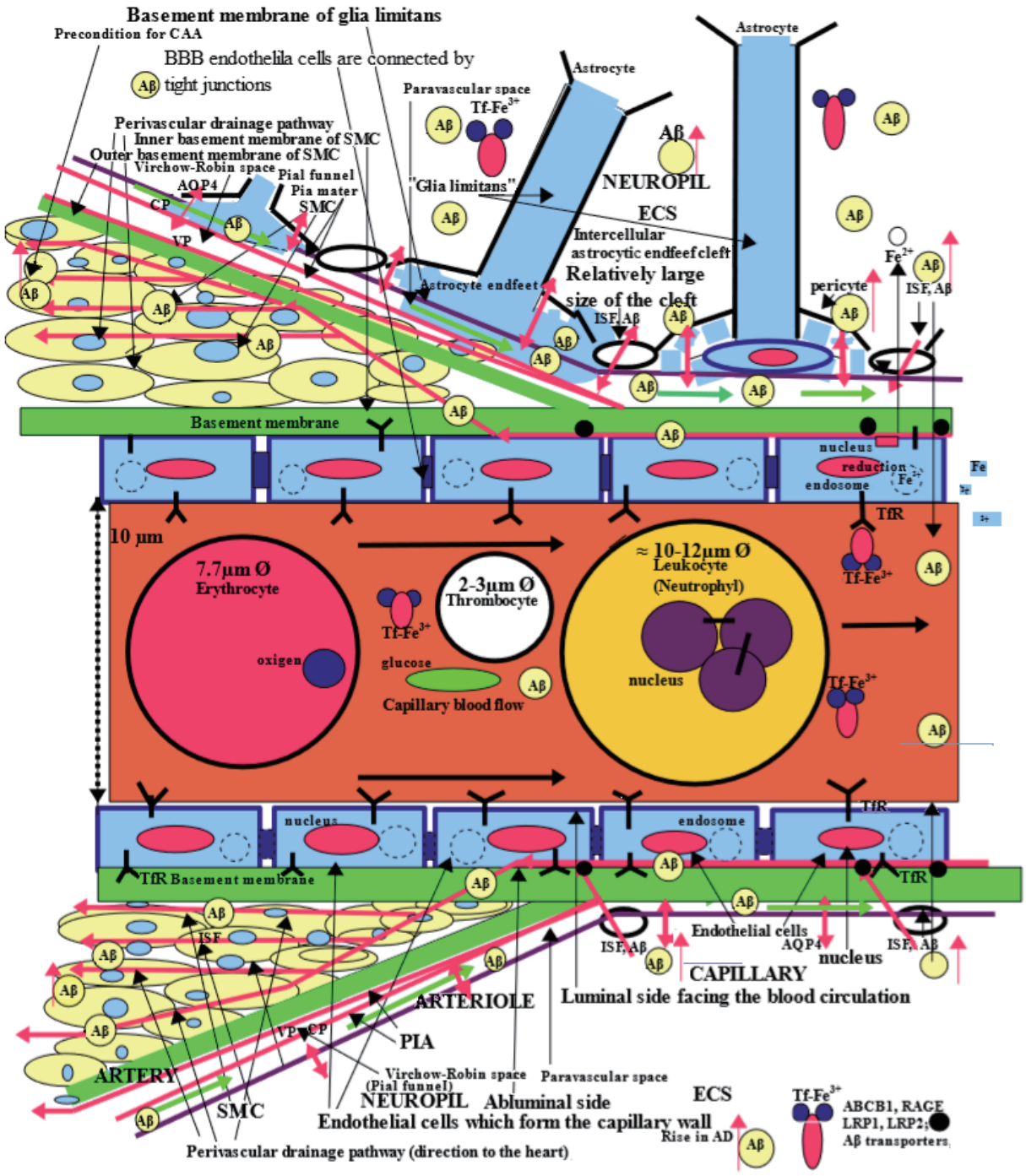

Fig. 4. **Schematic presentation of the blood brain barrier (BBB).**

Intercellular astrocytic endfeet cleft is permeable for nearly all proteins; "glia limitans", astrocytic endfeet processes; neuropil, extracellular space (ECS) in the brain cortex, composed of unmyelinated axons, dendrites and glial cell processes; ABCB1, RAGE, LRP1 and LRP2 are Aβ transporters.

AD, Alzheimer's disease; Aβ, amyloid beta; BBB, blood brain barrier; CAA, cerebral amyloid angiopathy; ISF, interstitial fluid; ECS, extracellular space; Tf, transferrin; Fe^{3+}, ferric iron; SMCs, smooth muscle cells; ●, ABCB1, ATP-binding cassette subfamily B member 1; ATP, adenosine triphosphate; AQP4, aquaporin-4 channel; CP, cortical pia; VP, vessel pia; RAGE, receptor for AGEs, AGEs, advanced glycation end products; LRP, low-density lipoprotein receptor-related protein; Ø, diameter.

pia and arachnoidea. The end of the route is in the cervical lymph nodes (***Fig. 5***) [15, 16, 21, 22].

Owing to the recent study by Louveau A *et al.* [23], the up to the resent dominant opinion about the brain, not having its own lymphatic routes, has drastically changed. The mentioned authors have discovered the functional lymph vessels slightly attached to the dural sinuses and able to transport CSF proteins and immune cells. By this discovery, they have made it possible to explain one of the, until now, unknown routes of waste products drainage out of the brain. The meningeal lymphatic route begins in the region of both eyes, passes out from the orbital cavity into the cranium, and extends along the superior sagital sinus. These vessels show typical molecular hallmarks of the lymphatic endothelial cells. They carry fluid (waste, cells, Aβ, Tf-Fe^{3+}) that has entered into them from the SAS. At their end they enter the deep cervical lymph nodes. In any case, this discovery has a great importance for the explanation of some links in the AD pathophysiology. The investigations indicate that the lymphatic CSF absorption declines during ageing (***Fig. 6***) [15, 20].

Sokolowski W *et al.* [24] also emphasize that, during a long period of time, the CSF circulation through the AG was considered as the dominant drainage route of waste products. Recent investigations dismiss this opinion and show that a significant quantity of CSF is drained through the lymphatic system. Disturbances of this drainage have a great impact in the developement of many neurodegenerative diseases. The authors have also compared the relation of these events in the great group of mammalia.

Moreover, researchers have demonstrated that a great part of CSF is drained by the axon extensions of the first cerebral nerve (olfactory nerve), which pass through the cribriform plate and with their endings contact the lymphatic system. It has been established that the olfactory nerve filaments enter the nasal mucose in the roof of the nasal cavity. On this place the CSF drains from the perineural subarachnoid space into the extracellular matrix, where it is absorbed by the lymphatic capillaries with blind endings. The route further leads up to the regional lymph nodes connected with the nasopharynx. All these facts indicate that the Aβ is in this way also cleared (***Fig. 4-5***) [15, 22].

Aβ degradation by proteases

Spies PE *et al.* [22] explain the event of decreased concentration of Aβ in the CSF in AD patients. The cause for this event is probably the extracellular aggregation of Aβ in the infusible plaques, which obstruct the transport of this peptide from the ISF into the CSF. The authors also emphasize the Aβ degradation by proteases, the Aβ intake by microglia, and its cleaning from ISF through BBB into the blood (importance of LRP).

However, without entering a detailed analysis of the process of intracellular and extracellular Aβ degradation, this study presents only a short insight into these events. The intracellular processes include the degradation by proteasome through the ubiquitin-proteasome route, lysosomal cathepsin enzymes, and thiolmetalloendopeptides which especially degrade Aβ monomers. Connected with extracellular degradation, mentioned are also neprilysin (a membrane-anchored zinc metalloendopeptidase), matrix metalloproteinases 2,3 and 9, and glutamate carboxypeptidase II. All these processes are damaged during the ageing process and in AD [15].

The examination of the choroid plexus indicates that it is the main place of the CSF production. This plexus is constructed from the net of capillaries surrounded by specialised epithelial cells called ependimal cells. These cells abound in small protrusions called cilia, and are critical for the CSF production. They filter water and many other substances by their transport into the ventricular space. These cells have an important protective function by selectively blocking the entrance of a lot of harmful compounds into the brain. Capillaries are fenestrated with pores about 70 nm broad. Blood enters the pore through the choroid artery and exits through the choroid vene. Besides the function of CSF production, the plexus is also a critical place for the thorough cleaning of Aβ present in the CSF, as well as a number of other disolved substances. Among these, especially important is the cleaning of free unbounded Fe^{2+} ions across the BCSFB. The DMT1-mediated transport mechanism is crucial for this event. The plexus alterations are connected with the ageing process and AD. They evidently disturb these cleaning processes. Investigations indicate that during alterations in the plexus occurs the drop in the CSF production and Fe^{2+} cleaning, the decline in the filtration, calcification, fibrosis, and Aβ deposition. The Aβ cleaning drops. The decline in the absorption in the capillary blood results in Aβ and iron ions accumulation in the brain, with the induction of dangerous pathologic events (***Fig. 7***) [25, 26].

Aβ transporters

The process of Aβ bilateral passage across the BBB is enabled by a number of transporters. Ageing and AD also lead to the Aβ passage alteration. Experiments indicate that Aβ crosses through the BBB from the extracellular space (ECS, ISF) into the blood stream. During these events low-density lipoprotein receptor-related protein-1 (LRP1) as the main transporter has a crucial role, and ATP-binding cassette subfamily B member 1 (ABCB1), α2-macroglobulin (α2M), LRP2 (megalin), ApoE-Aβ interaction, and oxidative stress (marked alteration) are also very important in these processes [15, 27].

In AD, the perivascular drainage is drastically damaged. The reasons for this event are linked with *APOE*ε4*, sedimentation of immune complexes, aging, and alterated arterial pulsations. A number of investigations confirm such explanation of *APOE*ε4* effects, otherwise linked with LOAD. This gene codes the ApoE isoform which is markedly less effective in the mediation of Aβ clearance as to other ApoE isoforms. Otherwise, ApoE protein, by the influence of ABCA1 (important Aβ transporter located on the abluminal side of the cerebral capillary endothelium), is subjected to lipidation, which favours ApoE-Aβ interaction in the perivascular space, with a consequently better possibility of Aβ transport by LRP1 or ABCB1 (also important Aβ transporter). LRP1 expression in AD is significantly reduced. Again it is necessary to emphasize that the *APOE*ε4* is a very strong risk factor for the occurence of AD. Related to its isomers, the genetic risk for AD shows the following relation: *APOE*ε4 > APOE*ε3 > APOE*ε2*. The presented data clearly indicate the reason for the strong Aβ drainage alteration, Aβ sedimentation and the concomitant sedimentation and blockade of iron ions efflux (***Fig. 7***) [15].

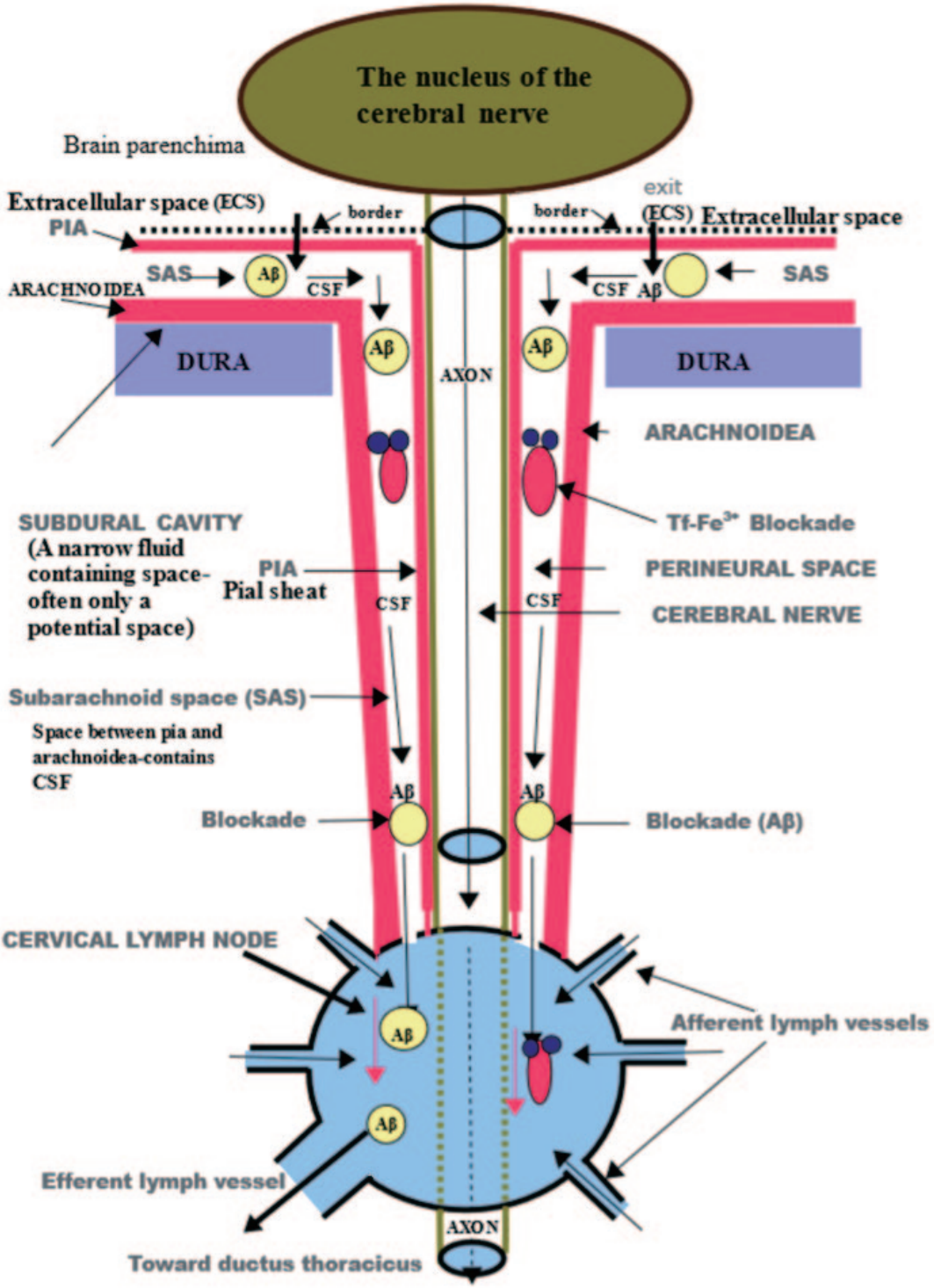

Fig. 5. **Schematic presentation of the perineural drainage pathway.**

Cerebral nerves exit from the nerve nuclei surrounded with the pial sheat. They pass firstly through the SAS, and then through the perineural space up to the cervical lymph nodes. CSF, Aβ, Tf-Fe^{3+}, and waste enter the nodes and axon continues its way up to the target organs. Lymph nodes have a number of afferent lymph vessels and one efferent vessel.

Aβ, amyloid beta; CSF, cerebrospinal fluid; SAS, subarachnoid space; ECS, extracellular space; Tf, transferrin; Fe^{3+}, ferric iron;

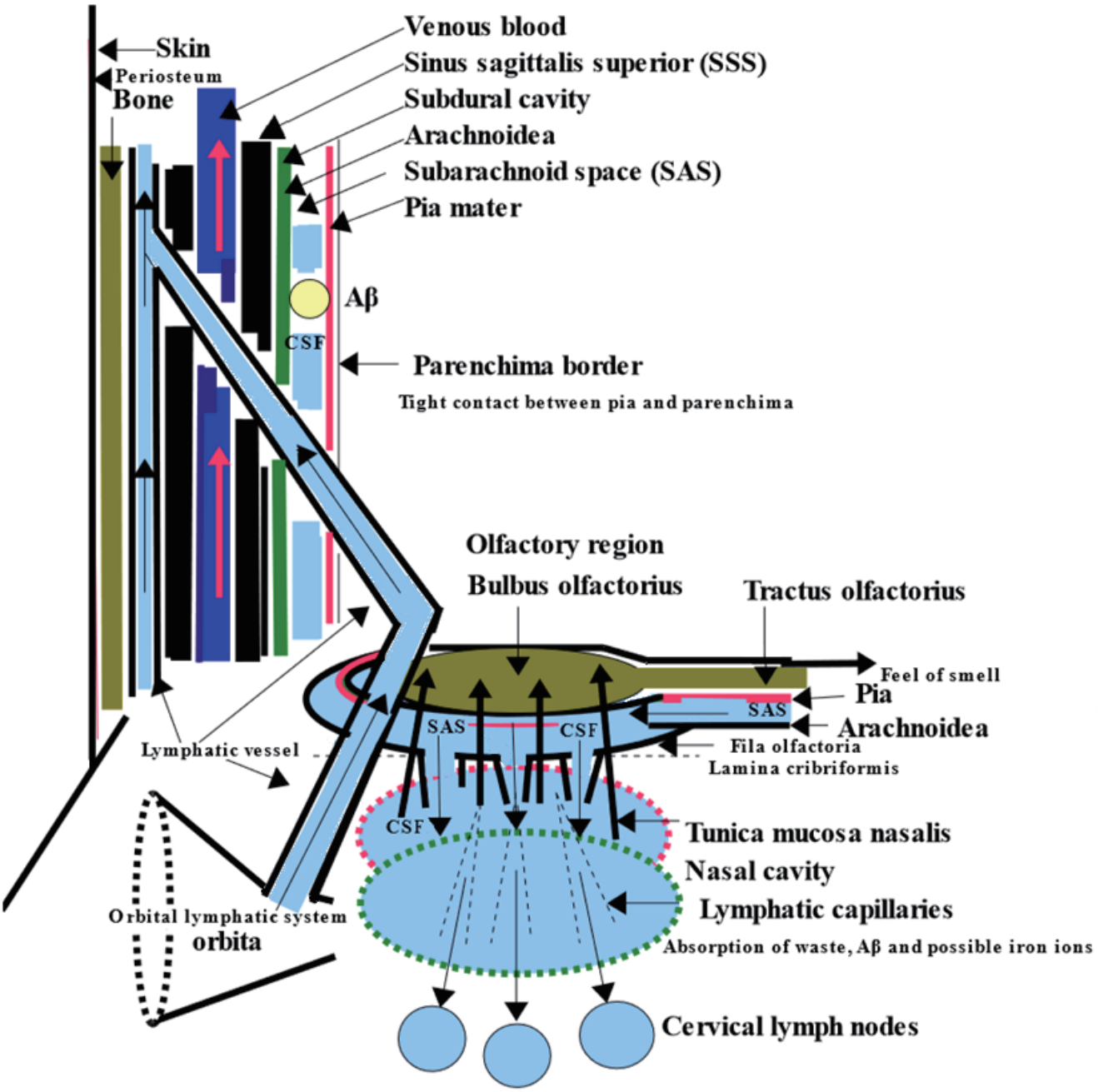

Fig. 6. **Schematic presentation of the two lymphatic drainage routes.**

There are visible two crucial lymphatic drainage routes; both transport waste, Aβ, and possible iron ions. Their origins are in the lymphatic systems of orbital and nasal cavities. The final targets of both pathways are the deep cervical lymph nodes.

Aβ, amyloid beta; SSS, sinus sagittalis superior; SAS, subarachnoid space; CSF, cerebrospinal fluid.

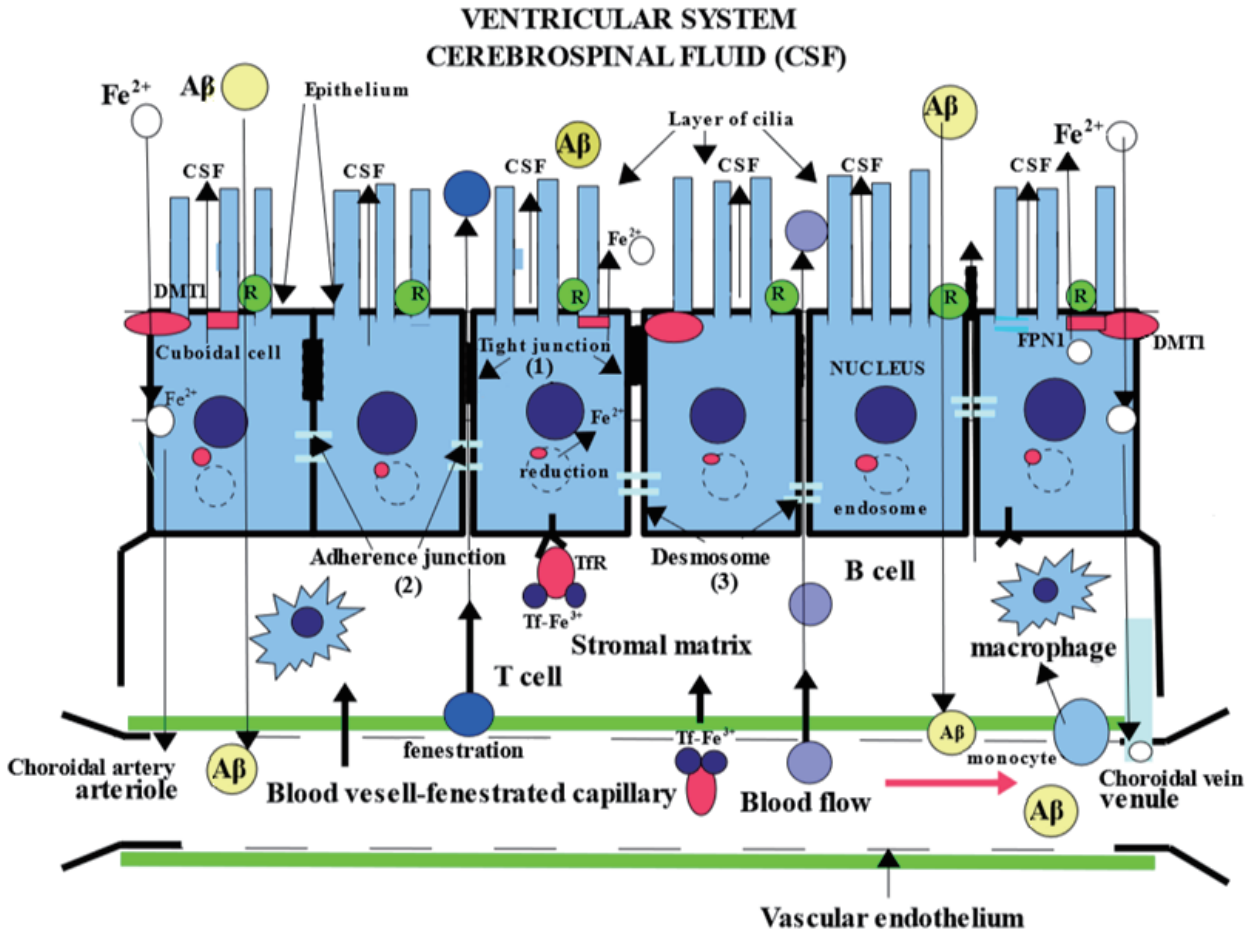

Fig. 7. **Schematic presentation of the blood cerebrospinal fluid barrier (BCSFB) choroid plexus.**

In AD, choroid plexus is affected by calcification, fibrosis and Aβ deposition. In AD and ageing, the CSF production by this plexus is decreased. There also occur the Aβ reduced clearance, and great disturbances in activity of LRP1, LRP2, ABCB1. There are three types of junctions between neighbouring epithelial cells: tight junction (1), adherence junction (2), and desmosome (3). Cilia are important for CSF flow. Ferric reductase, the enzyme located in the wall of the endosome, reduces Fe^{3+} to Fe^{2+}. Endosome is a smooth sack within the cell. Desmosome is a circular dense body located between two neighbouring epithelial cells, specialized for cell-to-cell adhesion.

AD, Alzheimer's disease; Aβ, amyloid beta; CSF, cerebrospinal fluid; SAS, subarachnoid space; ECS, extra-cellular space; DMT1, divalent metal transporter 1; Fe3+, ferric ion; Fe^{2+}., ferrous ion; Tf, transferrin; ▬, FPN1; ferroportin 1; ●, ferric reductase; R, receptors, *i.e.* LRP1, LRP2, ABCB1, or RAGE; LRP, low-density lipoprotein receptor-related protein; ABCB1, ATP-binding cassette subfamily B member 1; RAGE, receptor for AGEs; AGEs, advanced glycation end products.

Iron drainage

Aβ, abundantly present in the cerebral extracellular space of diseased AD patients, is transported by ISF bulk-flow clearance into the CSF, otherwise the perivascular space. Through this space it is transported into the lymphatic system. Aβ is also transported up to the lymphatic system through the perineural space. The damages are practically the same [13].

It is evident that the damage of the perivascular and perineural routes induce a decreased CSF drainage, and consequently the decline of the drainage of a number of waste products, among them iron ions. The mentioned decline of the drainage induces the consequent elevation of iron concentration in the ECS with its elevated reduction into the redox-active toxic ferrous ion, Fenton reaction and accelerated *OH generation.

The undertaken measuring of the ferritin concentration in the SAS, where it is the main iron ions transporter, indicates that in AD this concentration is elevated. Ferritin, otherwise the main iron storage, represents in fact the total quantity of iron in the brain, which coincides with its elevated values in SAS of AD patients, and the accelerated course of the disease. Tf concentration in the CSF is markedly low (decreased production by oligodendrocytes), so it is evident that the CSF transport is limited by this route. This indicates that ferritin is very important for the iron drainage from the brain (unfortunately, the drainage is disturbed when

the drainage routes are blocked by Aβ sedimentation). It is necessary to emphasize that ferritin is an effective iron transporter in any body region where iron is required. A number of studies are included in the measurement of iron concentration in the SAS, and in healthy people its values are very low [7, 28].

In AD, all the routes of iron (Fe^{2+}, Fe^{3+}) drainage, due to the Aβ pathologic accumulation, are alterated and iron does not have a good possibility to drain. At the same time the Aβ intake into the brain is normal. Due to the pathologic Aβ sedimentation, iron drainage is suppressed, its intracerebral concentration elevates, and Fenton reaction accelerates. The result is the general acceleration of patophysiological events. It is logical that an adequate intensive chelation therapy can diminish the unfavorable harmful effects of iron ions [4].

Future therapy for Aβ clearance

Actually, there exists a standard, but not yet an effective and satisfying therapy. There exist two groups of medicaments. The first group consists of cholinesterase blockaders: Aricept (donepezil hydrochloride), Axelon (rivastigmine), and Raminyl (galantamine hydrochloride). The second group includes N-methyl-D-aspartate (NMDA) receptor (NMDAR) antagonists, as well as Ebixa (memantine). The first group functions by blocking acetylcholine (Ach) esterase (Ach breaker). Memantine functions as NMDAR (N-methyl-D-aspartate receptor, glutamate receptor) blockader, and by this action protects the brain cells from the harmful effects of, in AD, elevated concentrations of released glutamate from damaged brain cells [1].

It is also necessary to mention some facts about, in AD, the elevated Aβ concentration in the brain interstitium. The logical task is to find the effective way of reducing this concentration. There are several possibilities to do this. Among them, one possibility is to reduce the Aβ production, and another is its effective and harmless degradation.

Cho JE *et al.* [29] emphasize that in AD, the aggregation of Aβ, resulting from the APP proteolytic cleavage, is the crucial event responsible for the pathophysiological cascade and consequent neuronal death. Actual medicaments for AD treatment can only alleviate symptoms rather than modify the underlaying molecular cause of AD. Recent therapeutic strategies, according to the authors, include the reduction of Aβ synthesis, inhibition of Aβ aggregation, immunotherapeutic enzymatic clearance of Aβ, targeting other amyloidogenic proteins interacting with Aβ, and amelioration of Aβ downstream toxic effects.

vanDyck CH [30] emphasizes that the accumulation of Aβ is the crucial link in AD pathophysiology. A great part of the potential modifying treatments for the disease actual in recent years, are directed against Aβ, including inhibitors of enzymes gamma secretase, beta secretase, and Aβ aggregation. However, the most used is immunotherapy, including both, active vaccines and passive immunization. Active immunization ensures consistant antibody titres and a good control of adverse events by stopping the treatment. During the past decade several mAβs (monoclonal) have been engineered to bind and clear Aβ. It is necessary to develop and improve this treatment in the near future.

Xiang Y *et al.* [31] in detail elaborate the capacity of peripheral tissues and organs in clearing brain-derived Aβ, suggesting that Aβ can be cleared in the periphery. They emphasize several pathways responsible for the brain Aβ efflux into the pherifery: transport across the BBB mediated by LRP1, drainage via perivascular and glymphatic pathway, flow through the AG, *i.e.* AV, the passage across the BCSFB, and the passage across the perineural and the lymphatic system. Experiments with mice have demonstrated that the main places for Aβ clearance are in the liver and kidney, and that the brain derived Aβ is cleaned during the passage across the capillary system of perifer organs and tissues (liver, kidneys gastrointestinal tract, and skin). It is established that the physiologic Aβ cleaning to a great extent contributes to the decline of the Aβ accumulation in the brain as well as of AD progression. The failure of the mentioned peripheral cleaning contributes to the AD occurence, especially in the case of kidney failure. Drugs with direct effect on the peripheral Aβ can have a favourable effect on the AD course, although they can not pass across the BBB. This refers to ApoE which during its circulation in the blood also can not enter the brain, but strongly elevates its efflux. At any rate, it is important to explore further possibilities of different drugs in the treatment of extracerebral Aβ which can slow down the course of AD. The authors even give preference to the peripheral cleaning of Aβ and not to the central cerebral cleaning. All the mentioned facts can effectively prevent AD pathogenesis, and can also be a valid therapeutic approach for AD. However, this approach demands yet a number of future investigations.

Conclusion

Alzheimer disease is a severe, chronic and lethal neurodegenerative disease connected with ageing and age. Due to the relative and absolute growth of the worldwide old population, its prevalence and incidence have a dramatic rise. The complex polygenetic etiology of this disease is not yet completely explained. The crucial factor in its pathology and patophysiology is the intracranial accumulation of Aβ peptide and iron ions. This peptide induces the reduction of non-toxic redox inactive ferric ion into the toxic redox active ferrous ion, with the consequent Fenton reaction and generation of the very aggressive and harmful compound hydroxyl radical (*OH). The results are dramatic irreparable damages of vital cerebral structures. The alterations of iron and Aβ drainage routes lead to the strong abundant intracerebral iron accumulation and to the accelerated AD pathophysiology. All the facts indicate, that presently, due to the practical impossibility of cleaning the mentioned drainage routes, the most effective therapy is the chelation therapy, especially using iron chelators.

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Conflict of interest

The authors claim no conflict of interest in this study.

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