ULTRASTRUCTURAL MALFORMATIONS OF THE CEREBRAL MICROVESSELS IN PATHOLOGICAL CONDITIONS – AN ELECTRON MICROSCOPIC STUDY

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Summary of Ph.D. Thesis

Department of Anatomy, Histology and Embryology
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Experimental and Clinical Neuroscience Doctoral Programme
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LIST OF ABBREVIATIONS

1 VO	one-vessel occlusion
2 VO	two-vessel occlusion
Aβ	beta-amyloid
AD	Alzheimer's disease
ANOVA	analysis of variance
apoB-100	apolipoprotein B-100
BBB	blood-brain barrier
BM	basement membrane
CBF	cerebral blood flow
CNS	central nervous system
FWM	frontal white matter
i. m.	intramuscular
i.p.	intraperitoneal
LDL	low density lipoprotein
L-NAME	*N*(G)-nitro-L-arginine-methyl-ester
LSD	least significant difference
NO	nitric-oxide
NOS	nitrogen-monoxide synthase
PWM	parietal white matter
Tg	transgenic
TNFα	tumor necrosis factor-alpha
VEGF	vascular endothelial growth factor
VLDL	very low density lipoprotein
Wt	wild-type
WM	white matter

1. INTRODUCTION

1.1. General architecture of the brain capillaries in healthy and pathological conditions

The cerebral capillaries have unique ultrastructure, which forms and serves the blood-brain barrier (BBB). There are three main cellular components of the BBB; the endothelial cells of the capillaries, special connective tissue elements called pericytes, and finally astrocytic endfeet surrounding the capillaries. The cellular units are surrounded by an accessory basement membrane (BM). For the occlusion and the prevention of paracellular transport, in between the contact points of the adjacent endothelial cells, tight junctions appear, which form the morphological basis for BBB. The BBB is a specialized system of the cerebral capillaries that protects the brain from harmful substances in the blood stream, while supplying the brain with the required nutrients for proper function. Unlike peripheral capillaries that allow relatively free exchange of substance across / between cells, the BBB strictly limits transport into the brain through physical, metabolic and enzymatic barriers. BBB breakdown is thought to be a key component in central nervous system (CNS) associated pathologies.

The morphological features of brain capillaries can be best studied with electron microscopy. Under various pathological conditions, the cerebral capillaries display typical malformations. In aging, or cerebral ischemia, the apical surface of the endothelial cells appear irregular, display microvillus-like processes into the lumen, and empty vacuoles may form occasionally. Pericytes often show type IV collagen and dense body (lysosomes) accumulation in their cytoplasm. Damage or disruption of the BBB causes swelling of the astrocytic endfeet, which typically appears as hypodense pericapillary areas in electron microscopic images. The most common ultrastructural deviations of the BM concern the accumulation of extracellular matrix proteins, which results in BM thickening and/or fibrous collagen deposition.

1.2. Cerebrovascular and other risk factors for Alzheimer’s disease

Sporadic (i.e. not genetically inherited) Alzheimer’s disease (AD) is considered as a multifactorial, progressive neurodegenerative disorder. In addition to amyloid toxicity and defect in tau-phosphorilation, various risk factors, such as cerebral hypoperfusion, neuroinflammation and hypercholesterolemia contribute to the development of AD (Fig. 1). Vascular risk factors are considered as major contributors to disease evolution and progression in AD. As such, moderate but persistent reduction in regional cerebral blood flow (CBF) compromises memory processes and contributes to the development and progression

of dementia. The association of decreased CBF (particularly in the temporal and parietal cortices) with AD has been firmly established, and the degree or pattern of cerebral hypoperfusion in mild cognitive impairment has been proposed as a predictive marker for the progression to AD. Persistently low baseline CBF has been proposed to induce ultrastructural pathology of the cerebral microvessels (Fig. 1). The BBB displays hypoperfusion-related ultrastructural abnormalities in the form of BM thickening and fibrous collagen deposits, which develop chronically during aging and dementia. The accumulation of collagen fibers in the microvascular BM may hinder specific BBB transport for important nutrients such as glucose and essential amino acids. In the aging brain, a significant correlation has been established between collagen deposits in the microvascular wall and advancing age in the frontal and occipital white matter (WM). Whether such BM pathology is related to cerebral hypoperfusion has been tested in rats, of which the common carotid arteries were permanently occluded (2-vessel occlusion, 2VO). Electron microscopic examination revealed microvascular BM thickening and collagen deposits 14 months after 2VO onset, which were comparable to those seen in the human post mortem studies. The proportion of affected capillaries in the hippocampus in 2VO rats was almost twice that in the controls. Based on the above findings, chronic cerebral hypoperfusion is suggested to be a causative, accelerating condition for ultrastructural BBB damage, which is also the focus of our current investigation.

1.2.1. Effects of the neuroinflammation on AD

Inflammation has also been proposed to be involved in the pathogenesis of AD. For instance, the expression of an innate pro-inflammatory cytokine profile in middle age appeared as an early risk factor of AD in old age. According to a widely held view, beta-amyloid (Aβ) deposits in the brain parenchyma activate microglia, which, in turn release pro-inflammatory cytokines and reactive oxygen species. This chronic cascade may eventually lead to neuronal damage. While inflammation within the brain is thus thought to be a potentially major neurodegenerative process, links between AD and inflammation in the periphery have also been suggested. As such, peripheral blood mononuclear cells produce higher levels of pro-inflammatory cytokines upon stimulation in mild cognitive impairment and early AD. However, no association between circulating inflammatory mediators and cerebromicrovascular injury that occurs in AD has been investigated. Here we set out to characterize ultrastructural BBB damage after intracarotid infusion of the pro-inflammatory cytokine, tumor necrosis factor-alpha (TNFα) in the rat, and to define whether nitric oxide (NO) is a mediator of TNFα in this regard.

1.2.2. Effects of the hyperlipidemia on AD

High plasma lipid content is known to favor plaque formation. Incidentally, high dietary cholesterol intake increases, while the use of statins reduces the risk for AD. Hypercholesterolemia caused by elevated plasma concentration or abnormal metabolism of low density lipoprotein (LDL, an important carrier of cholesterol), accelerates atherosclerosis. Furthermore, recent evidence suggests that the triglyceride-rich very low density lipoprotein (VLDL) also contributes to atherogenesis possibly through the inflammatory activation of vascular or foam cells. Apolipoprotein B-100 (apoB-100) is a constant surface component of both the cholesterol carrier LDL, and the triglyceride-rich VLDL in circulating blood plasma. It plays a pivotal role in VLDL assembly in the liver, and binds to specific receptors on the cell membranes to direct the lipoproteins to their proper metabolic sites. In atherogenesis, apoB-100 assembles atherogenic lipoproteins and acts as a mediator in the interaction between LDL and proteoglycans in the vascular wall, thereby promoting lipoprotein retention in the vascular intima. The elevated production or decreased removal of apoB-100-containing LDL from plasma has been associated with an increased susceptibility for atherosclerosis. In AD, apoB-100 was found up-regulated in the serum as shown by a proteomics study surveying potential plasma biomarkers for AD.

Genetically engineered mice expressing human apoB-100 (Tg*(apoB-100)*) were generated in order to model hyperlipidemia with increased serum cholesterol or triglyceride concentrations similar to human conditions, and to investigate related cardiovascular pathologies in experimental models. The established cardiovascular pathology of Tg*(apoB-100)* mice prompted us to explore potential, ultrastructural microvascular abnormalities in their brains, which may be relevant for the evolution of cerebromicrovascular injury AD.

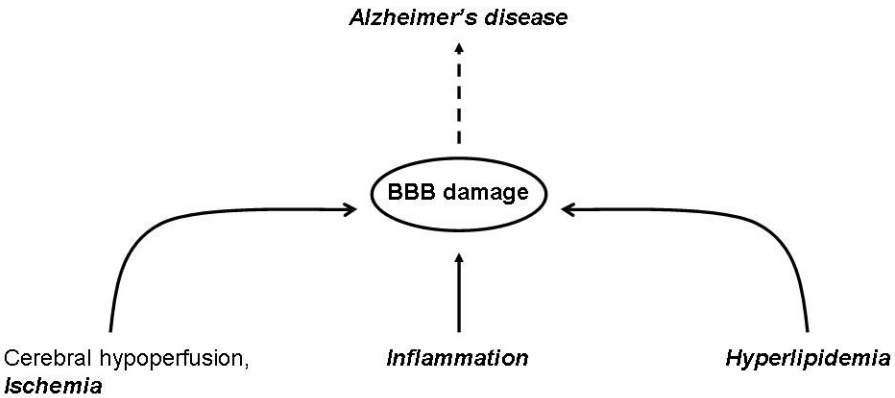

Figure 1. Various risk factors, considered in this study, which are expected to cause blood-brain barrier (BBB) damage that may contribute to Alzheimer's disease. The focus of our investigations was the ultrastructural malformations of the BBB. Conditions that were investigated in various experimental paradigms are highlighted in ***italics***.

2. OBJECTIVES

The general aim of our study was to describe the possible ultrastructural aberrations of the cerebral microvasculature in various pathological conditions. The disease processes specified below all occur – alone or in combination - in AD with ischemic components.
First, we sought to determine whether:

a) the microvasculature of the cerebral periventricular WM is injured in Alzheimer's disease;
b) the potential microvascular damage affects all WM areas (i.e. frontal, parietal, occipital) equally.

Second, we set out to:

a) investigate the effect of systemic inflammation (e.g. high level of the circulating proinflammatory cytokine TNFα) on the ultrastructure of the blood-brain barrier;
b) determine whether NO is a mediator of the expected TNFα-induced alterations in blood-brain barrier ultrastructure.

Third, we aimed to investigate whether hyperlipidemia:

a) causes cerebral microvascular lesions in itself;
b) augments ischemia-related capillary damage.

3. MATERIALS AND METHODS

3.1. Effects of normal aging and Alzheimer's disease on cerebral white matter microvessels – a human *post mortem* study

Post mortem periventricular WM samples of 17 patients were collected at autopsy from the frontal, parietal and occipital lobes, and kindly provided by Dr. Géza G. Kovács (now at: Institute of Neurology, Medical University Vienna, Vienna, Austria). The tissue blocks (1x1x1 cm) were immersed in Karnowsky fixative (2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4) and stored in the same solution until further processing. The neuropathological evaluation focused on the Braak staging indicative of the progression of Alzheimer's disease, and cortical Lewy body pathology also associated with dementia. Focal, mild atherosclerosis in the circle of Willis was labeled when yellow small plaques appeared in the vascular wall. Peripheral vascular risk factors have been also assessed. The degree of peripheral atherosclerosis was determined by examining the aorta, the carotid bifurcation and coronary arteries.

3.2. Effects of circulating TNFα on BBB ultrastructure – a rat study

Fifty male Wistar rats (280-350g) were anesthetized with 5% chloral hydrate (40 mg/kg i.p.); anesthesia was maintained continuously up to the end of the experiment. The right femoral vein and the right common carotid artery were cannulated for the infusion of solutions. Animals were pretreated (n=30) or not (n=20) with the nitrogen monoxide synthase (NOS) inhibitor *N*(G)-nitro-L-arginine-methyl-ester (L-NAME), at a dose of 20 mg/kg in 1 ml saline, given over 2 minutes intravenously. Fifteen minutes after the L-NAME infusion, TNFα (2.5 μg/kg, solubilized in 1 ml phosphate-buffered saline; human recombinant, Sigma) or 1 ml saline was administrated at a speed of 0.1 ml/min into the right common carotid artery with a laboratory pump. The animals were sacrificed by transcardial perfusion with saline followed by a solution of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1M phosphate buffer, 45 min, 4 h or 8 h after the end of TNFα or saline infusion. The brains were removed and stored in the same solution until further processing. Samples of the ipsilateral, frontoparietal cortex (Bregma 0.20 mm) were prepared for electron microscopy.

3.3. Effects of hyperlipidemia and/or ischemia on BBB ultrastructure – a mouse study

Transgenic and wild-type mice were generated, raised, characterized (i.e. serum lipid profile), and kindly supplied by the research group of Prof. Miklós Sántha (Laboratory of Animal Genetics and Molecular Neurobiology, Institute of Biochemistry, Biological Research Center, Szeged) and Dr. Tamás Csont (Cardiovascular Research Group, Department of Biochemistry, Faculty of Medicine, University of Szeged). Six-week-old Tg(apo-b 100) (n=23) and wild-type (Wt) mice (C5/B6, n=26) were fed for 17-19 weeks with standard laboratory rodent chow, or 2% cholesterol-enriched diet prepared by supplementing the standard diet with cholesterol. The animals received food *ad libitum* throughout the study; the body weight at the end of the dietary regime was similar for all experimental groups. Serum lipid levels were determined in blood samples to justify the development of hyperlipidemia. The mice were anesthetized with 3% chloral hydrate (0.015 ml/g i.p.) and injected with 0.05 ml atropine (0.1 mg/ml; i.m.) at 24 weeks of age. On all of the animals, unilateral global forebrain ischemia was induced by permanent occlusion of either common carotid artery (one-vessel occlusion, 1VO). The mice were transcardially perfused 24 h after the onset of 1VO with 50 ml 4% paraformaldehyde in 0.1 M phosphate buffer (pH=7.4) and the brains were removed. The frontoparietal cortex was postfixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer for 1 week. Samples of the ipsilateral and contralateral frontoparietal cortices (Bregma 1.34 mm) were dissected and routinely embedded for electron microscopic investigations as described below.

3.4. Electron microscopy

Tissue blocks were prepared for light and electron microscopic examination. Samples were dehydrated by increasing concentrations of ethanol, and embedded in Durcupan epoxy resin. Semi-thin sections were cut on an ultramicrotome and stained on object glasses with a 1:1 mixture of 1% methylene blue and 1% Azure II blue. The samples were then coverslipped with DPX and analyzed under a light microscope. Ultrathin sections were cut from the same blocks and collected in 200-mesh copper grids. The preparations were then contrasted with 5% uranyl acetate and Reynolds lead citrate solution. Finally, the samples were analyzed with a Philips TM10 transmission electron microscope. Photographs were taken with a computer-assisted digital camera (MegaView II, Soft Imaging Systems, Germany).

3.4.1. Determination of microvascular damage and quantitative analysis

All cortical layers were systematically scanned in the rodent experiments (rat: approximately 0.13 mm^2 tissue surface ~25±7 capillary cross sections; mice: about 0.14 mm^2 cortical area ~ 28±8 capillaries), and an entire section in the human study (approximately 0.49 mm^2 of sample surface ~8±4 microvessels) on a randomly selected sample grid.
First, the lumen diameter of microvessels was measured. Microvessels only of a defined lumen diameter were included in the analysis (i.e. rodent: d<7 μm, human d<12 μm). In case the vessel profile was not an exact cross section (circle) but slightly oval, the shortest diameter was taken. The diameters of all the investigated vessels in each sample were averaged, and this mean was used as a single value for further statistical analysis. Vascular density was expressed as the number of microvascular profiles on 1mm^2 surface.

In the rodent studies, capillaries displaying finger-like endothelial processes protruding the vessel lumen (microvilli) or swollen astrocytic endfeet were counted and the number was expressed as percentage of the total number of capillaries examined. The ratio of intact capillaries was also calculated and expressed as percentage.

In the human study, the analysis focused on BM pathology, which was noted when fibrous collagen deposition in the BM occurred. Another investigated pathological feature was the accumulation of perivascular fibrous collagen, surrounding the blood vessels. The number of small vessels with either of the above BM pathology was counted and expressed as percentage of the total number of microvessels examined.

The mean lumen diameters and the ratio of microvessels displaying perivascular fibrous collagen were correlated with the progression of Alzheimer's neuropathology defined with Braak staging, and were compared with the age of the subjects'.

3.4.2. Statistical analysis

In the rat study, a two-way ANOVA paradigm of the software SPSS 12.0 was used (variables: treatment and survival time). In the mouse study, data were analyzed with a three-way ANOVA for genotype (Tg(apoB-100) vs. Wt), diet (cholesterol-enriched vs. standard) and ischemia (ipsilateral vs. contralateral cortex). In both rodent experiments, ANOVA was followed by a Fisher least significant difference (LSD) post-hoc test for group comparisons. In the human study, statistical analysis was performed with the non-parametric Mann-Whitney U-test, and correlation analysis was performed with a Pearson's one-tailed correlation test. Results were considered to be significantly different at a probability level of $p<0.05$* and $p<0.01$**.

4. RESULTS

4.1. The ultrastructure of cerebral WM microvessels in normal aging and Alzheimer's disease

In all study groups, massive fibrous collagen deposition was observed around some microvessels, either associated with the basement membrane, or extending deep into the perivascular space. In healthy age-matched controls, only about 20% of the investigated vessels displayed perivascular collagen deposition; most microvessels and their outskirts were devoid of fibrous collagen bundles. In contrast, the ratio of affected microvessels increased with the progression of Braak neuropathology, particularly in the frontal and parietal WM (FWM and PWM, respectively): the values increased from 20% to 71% in the FWM, and from 19% to 67% in the PWM. In order to evaluate the contribution of aging to BM pathology, perivascular collagen deposition was related to age. Increasingly more microvessels displayed perivascular collagen bundles with advancing age in the FWM, but not in the PWM. The average microvascular lumen diameter was calculated to be 5 μm in healthy age-matched controls and in the early stages of Braak neuropathology. The lumen diameter increased to an average of 6.6 μm in the PWM of cases with Braak stage III-IV, and V-VI. In addition, a positive, linear correlation appeared between lumen diameter and age in the PWM.

4.2. Effects of circulating TNFα on blood-brain barrier ultrastructure

In the semithin sections, perivascular edema was apparent around the arterioles in the brain parenchyma of the TNFα treated animals. At electron microscopic level, the endothelial

cells of cerebral microvessels exhibited the following discernible abnormalities: (i) the apical surface of the endothelial cells displayed finger-like projections protruding into the lumen, and (ii) the endothelial cytoplasm contained hypodense vacuoles. The astrocytic endfeet surrounding the microvessels were swollen as shown by their low electron density and enlarged area covered. Finally, the extensive swelling of the astrocytic endfeet occasionally deformed the capillary lumen. According to the outcome of the quantitative analysis, TNFα infusion itself increased the ratio of capillaries with swelling of the astrocytic endfeet more than twofold at 45 min, compared to the control (48% and 22%, respectively), and a further TNFα-related increase was observed as time progressed (65% at 8 h). In contrast, the ratio of capillaries with astrocytic swelling was reduced remarkably by L-NAME pretreatment, from 48% to 18% at 45 min, from 61% to 34% at 4 h, and from 65% to 51% at 8 h after TNFα infusion. Although the statistical analysis has not revealed any significant changes in the lumen diameter of capillaries between experimental groups, both TNFα and L-NAME reduced microvascular diameter noticeably. In particular, TNFα alone reduced the lumen diameter from the control value of 4.56 μm to 4.08 μm at 45 min, which progressively decreased to 3.86 μm at 4 h, and to 3.61 μm at 8 h. After the treatment with L-NAME alone or in combination with TNFα, the capillary lumen diameter varied in the interval 3.51-3.99 μm irrespective of survival time, in contrast with the 4.56 μm for the saline control group.

4.3. Effects of hyperlipidemia and/or ischemia on BBB ultrastructure

Forebrain ischemia in the ipsilateral frontoparietal cortex with respect to 1VO markedly increased the ratio of cortical capillaries with pericapillary astrocytic swelling from 36.5±4.0% to 62.1±4.2% in Wt mice (dietary groups merged for values, contralateral vs. ipsilateral, respectively; $P<0.001$) and from 33.2±4.6% to 56.0±5.4% in Tg(apoB-100) mice (dietary groups merged for values, contralateral vs. ipsilateral, respectively; $P<0.003$.

The luminal endothelial surface appeared to be irregular, displaying microvilli, indentations, and occasional vacuoles. The ratio of microvessels displaying endothelial microvilli demonstrated no definite change, though a slight increase appeared in the ipsilateral cortex after 1VO when compared to the contralateral side (dietary groups merged for values: 20.1±2.3 vs. 14.3±1.6% in the Wt mice; 13.6±2.7 vs. 11.7±1.7% in Tg(apoB-100) mice, ipsilateral vs. contralateral, respectively). The ratio of capillaries devoid of any of the above pathology (intact microvessels) decreased from an average of 65.2±3.7% to 32.1±5.6% in Wt mice (dietary groups merged for values, contralateral vs. ipsilateral, respectively; $P<0.001$) and from 56.78±5.60% to 38.64±5.06% in Tg(apoB-100) mice (dietary groups merged for values, contralateral vs. ipsilateral, respectively; $P<0.004$) due to 1VO.

The microvascular integrity was not altered by either the transgenic genotype or the experimental diet.

Microvascular density appeared significantly lower in Tg(apoB-100) mice as compared with Wt mice (dietary groups merged for values: 195±7 vs. 223±8 vessels/mm^2; P<0.008), but was not consistently altered by 1VO (comparison between ipsilateral and contralateral cortices; P<0.708). The lumen diameter of the capillaries demonstrated an inverse pattern to the microvascular density. The lumen of cortical capillaries of Tg(apoB-100) mice was significantly more dilated as compared with Wt mice (dietary groups merged for values: 3.16±0.5 vs. 2.88±0.6 μm and P<0.001). A tendency in reduction of lumen diameter was observed in the cortex ipsilateral to 1VO as compared with the contralateral side in both Tg(apoB-100) and Wt mice on standard diet; however it did not reach statistical significance (P<0.129). The cholesterol-enriched diet exerted no detectable effect on capillary diameter (P<0.925).

5. DISCUSSION

Injuries of the cerebral microcirculation are hypothesized as either the main or contributing risk factors for the progression of pathophysiologic mechanisms leading to various disease manifestations, such as vascular dementia, AD, neuroinflammation, or hyperlipidemia. Therefore capillary degeneration in the above pathological states leads to serious functional consequences. First of all, the transport mechanisms through the BBB are hampered and the local regulatory mechanisms are less effective. The partial or complete breakdown of the BBB promotes the non-specific permeability of the BBB, while the facilitated transport of indispensable materials (e.g. glucose, amino acids) is damaged. The opening of the BBB favors different pathologic processes. From the circulatory system, toxic agents are able to traffic into the brain parenchyma, and nutrient supply becomes suboptimal. These processes contribute to cognitive dysfunction, and have been described to be part of the etiology of AD.

In order to maintain constant and optimal blood supply to the brain through a morphologically compromised cerebrovascular network, compensatory mechanisms must turn on. One possible way to manage normal flow is the adjustment of microvascular density in order for nutrient supply to meet the requirements of the nervous tissue. Vascular remodeling mediated by angiogenesis through vascular endothelial growth factor (VEGF) is considered as a potential mechanism to save brain areas jeopardized by suboptimal nutrient supply through damaged capillary walls. In addition to the extension of the microvascular network, other mechanism may also compensate for the hindered nutrient supply through a damaged microvascular system. Increased microvascular caliber may serve the maintenance of optimal basal blood flow.

5.1. Alterations of cerebral WM microvessels in Alzheimer's disease

The main goal of the study was to determine, whether the microvasculature of cerebral periventricular WM is injured in AD, and whether the possible microvascular damage appears in the frontal, parietal and occipital WM areas evenly. Our human study presented the following results. Perivascular fibrous collagen deposition almost linearly increased with advancing age in the FWM and microvascular lumen diameter displayed a linear, positive correlation with age in the PWM. In addition, perivascular fibrous collagen accumulation more than tripled in AD patients with neuropathology of Braak V-VI in the PWM.

The ultrastructural changes of microvessels shown here share similar characteristics with those found in capillaries in the cerebral cortex and, more interestingly, in the WM after chronic, experimental, cerebral hypoperfusion in rats. In the experimental model of chronic cerebral hypoperfusion, the typical capillary abnormalities included 25% higher occurrence of degenerative pericytes around blood vessels and 25% higher incidence of BM thickening, with or without fibrosis, in hypoperfused animals. These data demonstrate that decreased CBF induces ultrastructural damage of cerebral microvessels. In addition, experimental cerebral hypoperfusion that evokes ischemia has been found to have a deleterious impact on the medullary tissue. The injury appears in the form of glial activation and rarefaction of the WM. We, therefore, hypothesize that the microvascular abnormalities found in our pool of human samples may well be a consequence of chronic cerebral hypoperfusion.

We have additionally shown that the average size of microvascular lumen increases with the severity of AD, especially in the PWM. The increased lumen diameter is suggested to be a compensatory mechanism to maintain normal cerebral perfusion and transport in spite of the increased rigidity and thickness of the microvascular wall as demonstrated by massive collagen deposition.

Our data have demonstrated that the enhanced perivascular accumulation of collagen and the dilation of micorvascular lumen in the periventricular WM are region-specific. The most affected site was the parietal region, where the collagen deposition increased most intensely, and the microvascular lumen dilated most noticeably. The microvascular alterations presented here suggest a potentially increased vulnerability of the frontal and parietal areas. The susceptibility of the frontal and parietal WM to injury may be rooted in the angioarchitecture and perfusion of the region.

5.2. BBB ultrastructure in inflammatory processes

TNFα is a proinflammatory cytokine, which is produced by various cell types, e.g. macrophages and lymphocytes. TNFα is a well-known and potent vasodilatator in various

experimental *in vivo* models. The suggested pathway for the vasodilatory action of TNFα involves NO; NOS inhibitors successfully block the vasoactive property of the TNFα. In addition to the functional role of TNFα in cerebral blood flow regulation, TNFα can impose structural damage to the cerebral microvasculature. In our present study, the ultrastructural analysis of the cerebrocortical capillaries was performed after TNFα treatment in rats. The most obvious aberration concerned the pericapillary astrocytic endfeet, which appeared considerably swollen, seen around the capillaries as distorted, dilated areas with low electron density in electron microscopic images. Astrocytic swelling is a marked feature not only of inflammatory reactions, but also of seizure-related states. In line with our findings, intracarotid administration of TNFα in newborn piglets resulted in the opening of the BBB. The opening of the BBB in pathologic states can be clearly demonstrated macroscopically after intravenous Evans blue, or at light microscopic level by sodium fluorescein. The astrocytes have various roles in the induction of normal formation of brain capillaries, and in addition the clearance and degradation of Aβ plaques in AD, so functionally intact astrocytes may postpone the development of dementia. Taken together, if the astrocytic endfeet are swollen as shown here, the cross-talk with the endothelium becomes disturbed or discontinues, which can lead to BBB dysfunction. In support of this view, the BBB disruption indicated by the extravasation of Evans blue was associated with astrocytic swelling that involved aquaporin-4, a major water channel implicated in the formation of injury-related brain edema.

5.3. The ultrastructure of the BBB in hyperlipidemia and/or ischemia

Our investigation focused on whether hyperlipidemia (i.e. the expression of human apoB-100 in transgenic mice, and/or cholesterin-rich diet) (i) causes cerebral microvascular lesions, and (ii) whether it augments ischemia-related cerebral capillary damage. Comparison of the cerebral capillary network proved that the Tg(apoB-100) mice exhibited decreased density and increased capillary lumen diameter as compared with Wt mice. The density of the cerebral capillary network is established during early development, and dynamically reacts to environmental challenges and pathophysiological conditions. The expression of VEGF is hypothetised as an important determinant of angiogenesis during the development in the brain. In hyperlipidemic Tg(apoB-100) mice, the capillary density in the cerebral cortex is proposed to be lower due to their high plasma cholesterol or triglyceride profile, thus inhibiting angiogenesis possibly through hampered VEGF signaling. The increase of the lumen diameter is proposed as a complementary and compensatory mechanism in order to maintain standard cerebral perfusion rate despite a less dense capillary network in Tg(apoB-100) as compared with Wt mice.

Unilateral forebrain ischemia was induced by 1VO in Tg(apoB-100) and in Wt mice to compare the possible effects of the hyperlipidemia on the ultrastructure of cerebral capillaries. In the present study, the swelling of astrocytic endfeet and the presence of endothelial microvilli became more prominent in the ischemic hemisphere. These data agree with previous observations made in models of cerebral ischemia: (i) electron microscopic analysis of cerebral microvessels revealed compressed capillaries consistently surrounded by swollen astrocytic endfeet; and (ii) the widespread appearance of cerebral endothelial microvilli. These morphological features imply some harmful functional changes: astrocytic pathology compromises the integrity of the BBB, while endothelial microvilli might increase microvascular resistance, leading to moderate hemodynamic impediments.

The data presented here showed that hyperlipidemia in human apoB-100 transgenic mice and chronically elevated dietary cholesterol–alone or in combination–did not have any impact on capillary ultrastructure in the non-ischemic hemisphere, and did not exacerbate ischemia-induced microvascular lesions in the mouse brain. High serum lipid level-related, morphological, vascular lesions in experimental models are assumed to be confined to large-caliber arteries (e.g. aorta, carotid arteries), rather than cerebral capillaries. Cerebral capillary density reduced by high plasma lipid levels as presented here may affect cerebrovascular reactivity during pathophysiological challenges—ischemia being one of the most prominent types.

6. CONCLUSIONS

The studies presented here all aimed to investigate the fine ultrastructure of cerebral capillaries in various pathological conditions that appear as risk factors for AD. We have found that, with the exception of hyperlipidemia, the ultrastructure of the BBB becomes compromised in all these conditions, which is suggested to have functional implications. Since the BBB is the site of selective nutrient and waste product trafficking between blood and the nervous tissue, its structural integrity is crucial to fulfill its function and meet the metabolic demands of the CNS.

AD is a progressive mental disorder with undefined origin. It has been firmly established that cerebrovascular pathology and chronically reduced CBF are hallmarks of AD, cerebral hypoperfusion is a predictive marker of the disease, and that cerebrovascular risk factors contribute to the disintegration of cognitive function over the course of the disease. Our present study confirms the occurrence of microvascular injury in AD brains, and reveals conditions (i.e. ischemia, inflammatory processes), which are causative for the development of cerebral microvascular damage. Our results shed light on the complexity of causative elements that may add up to damage the BBB, and ultimately lead to memory dysfunction.

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LIST OF PUBLICATIONS, RELATED TO THIS THESIS

I. Fabene PF, Weiczner R, Marzola P, Nicolato E, Calderan L, Andrioli A, Farkas E, **Süle Z**, Mihály A, Sbarbati A: Structural and functional MRI following 4-aminopyridine-induced seizures: a comparative imaging and anatomical study.
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