Morphological and functional changes of the blood–brain barrier in hypertriglyceridemia

Ph.D. Thesis

Beáta Barabási

Supervisors:

Mária Deli, M.D., Ph.D, D.Sc

Zsófia Hoyk, Ph.D

Biological Barriers Research Group

Institute of Biophysics, HUN-REN Biological Research Centre, Szeged

Doctoral School of Theoretical Medicine, Albert Szent-Györgyi Medical School

University of Szeged

Szeged, Hungary

2024

LIST OF PUBLICATIONS

Publications related to the subject of the thesis:

I. Barabási B, Barna L, Santa-Maria AR, Harazin A, Molnár R, Kincses A, Vigh JP, Dukay B, Sántha M, Tóth ME, Walter FR, Deli MA, Hoyk Z. Role of interleukin-6 and interleukin-10 in morphological and functional changes of the blood-brain barrier in hypertriglyceridemia. Fluids Barriers CNS. 2023 Mar 7;20(1):15. doi: 10.1186/s12987-023-00418-3 ($IF_{2022-2023}$: 7.3, Journal Ranking: D1)

II. Hoyk Z, Tóth ME, Lénárt N, Nagy D, Dukay B, Csefová A, Zvara Á, Seprényi G, Kincses A, Walter FR, Veszelka S, Vígh J, **Barabási B**, Harazin A, Kittel Á, Puskás LG, Penke B, Vígh L, Deli MA, Sántha M. Cerebrovascular Pathology in Hypertriglyceridemic APOB-100 Transgenic Mice. Front Cell Neurosci. 2018 Oct 25;12:380. doi: 10.3389/fncel.2018.00380. (IF_{2018}: 3.9, Journal Ranking: Q1)

Publications not related to the subject of the thesis:

I. Barabási B, Csondor A, Martín-Pozas T, Pulupa Sánchez AM, Antalffy G, Siklós L, Gómez-Pinedo U, Párducz Á, Hoyk Z. Effect of axotomy and 17β-estradiol on P2X7 receptor expression pattern in the hypoglossal nucleus of ovariectomized mice. Neuroscience. 2016 Apr 5;319:107-15. doi: 10.1016/j.neuroscience.2016.01.049.
(IF_{2016}: 3.277, Journal Ranking: Q1)

II. Dukay B, Walter FR, Vigh JP, **Barabási B**, Hajdu P, Balassa T, Migh E, Kincses A, Hoyk Z, Szögi T, Borbély E, Csoboz B, Horváth P, Fülöp L, Penke B, Vígh L, Deli MA, Sántha M, Tóth ME. Neuroinflammatory processes are augmented in mice overexpressing human heat-shock protein B1 following ethanol-induced brain injury. J Neuroinflammation. 2021 Jan 10;18(1):22. doi: 10.1186/s12974-020-02070-2. (IF_{2021}: 9.589, Journal Ranking: Q1)

Cumulative impact factor: 24.066

LIST OF ABBREVIATIONS

APOB: Apolipoprotein B

AQP4: Aquaporin 4

BBB: Blood-brain barrier

BEC: Brain endothelial cell

BSA: Bovine serum albumin

CNS: Central nervous system

DHA: Docosahexaenoic acid

DMEM: Dulbecco's modified Eagle's medium

GFAP: Glial fibrillary acidic protein

GLUT: Glucose transporter

GSK3β: Glycogen synthase kinase-3 beta

IBA: Ionized calcium binding adaptor molecule

IL: Interleukin

LDL: Low-density lipoprotein

LOX: Lectin-like oxLDL (oxidized low-density lipoprotein) receptor

LRP: Low density lipoprotein receptor-related protein

MEOX2: Mesenchyme homeobox protein 2

MFSD2A: Major facilitator superfamily domain-containing protein 2a

NF-κB: Nuclear factor kappa B

PB: Phosphate buffer

PBS: Phosphate buffered saline

P-gp: P-glycoprotein

S100B: S-100 protein family calcium-binding protein B

SF: Sodium fluorescein

TEER: Transendothelial electrical resistance

TJ: Tight junction

TJP: Tight junction proteins

TNF: Tumour necrosis factor

VLDL: Very low density lipoprotein

WT: Wild type

ZO-1: Zonula occludens-1

1. Introduction

Hyperlipidemia, characterized by elevated levels of lipids in the bloodstream, is intricately linked to an array of systemic health issues, particularly cardiovascular and cerebrovascular diseases, and also contributes significantly to the pathogenesis of neurodegenerative disorders. Lipids, including cholesterol and triglycerides, due to their insolubility in water, are transported in the plasma within lipoprotein complexes. These complexes are structured with a hydrophobic core that contains the lipids, surrounded by a hydrophilic outer shell composed of phospholipids and proteins, making them soluble in blood. The protein component of these complexes, particularly apolipoprotein B-100 (APOB-100), is crucial for the stability of the complexes and their interaction with cellular receptors. APOB-100 is primarily associated with low-density lipoprotein (LDL) and very low-density lipoproteins (VLDL), which play key roles in cholesterol and triglyceride transport. Elevated levels of LDL are linked to the development of atherosclerosis, where arterial walls thicken due to the accumulation of lipids and immune cells, leading to plaque formation. This process is exacerbated by the oxidation of LDL particles within the subendothelial space, triggering a cascade of inflammatory responses. This inflammatory process involves the expression of cell adhesion molecules on endothelial cells, attracting monocytes that transform into macrophages and consume oxidized LDL, becoming foam cells that contribute to plaque stability and expansion. The progression from elevated lipid levels to atherosclerosis and the subsequent impact on the cerebral vasculature underscores the potential for systemic lipid dysregulations to impair brain functions and facilitate neurodegenerative changes, linking metabolic, vascular, and neurological health.

Macrophages and smooth muscle cells in atherosclerotic lesions produce interleukin (IL)-6, a pleiotropic cytokine, which is associated with endothelial dysfunction. Vascular cells may promote inflammatory processes by synthesizing tumour necrosis factor (TNF)-α, IL-1β, IL-6, IL-8 and IL-15, and may exert anti-inflammatory action mainly by transforming growth factor-β production. Another cytokine playing an anti-inflammatory role in atherosclerosis is IL-10, which is primarily produced by macrophages, T and B lymphocytes, and in very small quantities by brain capillary endothelial cells. Hyperlipidemia is associated with systemic inflammation even without cardiovascular pathologies. Patients with high triglyceride levels have an increased capacity to produce TNFα and IL-6. Systemic inflammation also affects the brain and its vasculature, and damages their functions.

In order to study the pathological alterations induced by hyperlipidemia, an animal model was established by the research group headed by Miklós Sántha, PhD, DSc, in 2005, in the Biological Research Centre. This animal model is a transgenic mouse line, which overexpresses the human APOB-100 protein in different tissues such as the liver, heart and brain. APOB-100 transgenic mice show significantly elevated serum triglyceride and cholesterol levels when fed with normal chow and cholesterol rich diet, respectively. Moreover, various neurodegenerative processes occur in the brain of hypertriglyceridemic APOB-100 transgenic mice. Widespread neuronal cell death and apoptosis of cortical and hippocampal neurons was detected in this model. APOB-100 overexpression is also linked to an increased level of lipid peroxidation in cortical and hippocampal brain regions and to impaired cognitive function. The observed pathological changes caused by hyperlipidemia are well-known characteristics of Alzheimer's disease. Several neurodegenerative processes, including Alzheimer's disease, Parkinson's disease and multiple sclerosis are related to impaired cerebrovascular functions, involving the dysfunction of a dynamic interface between the brain parenchyma and the circulating blood, known as the blood-brain barrier (BBB).

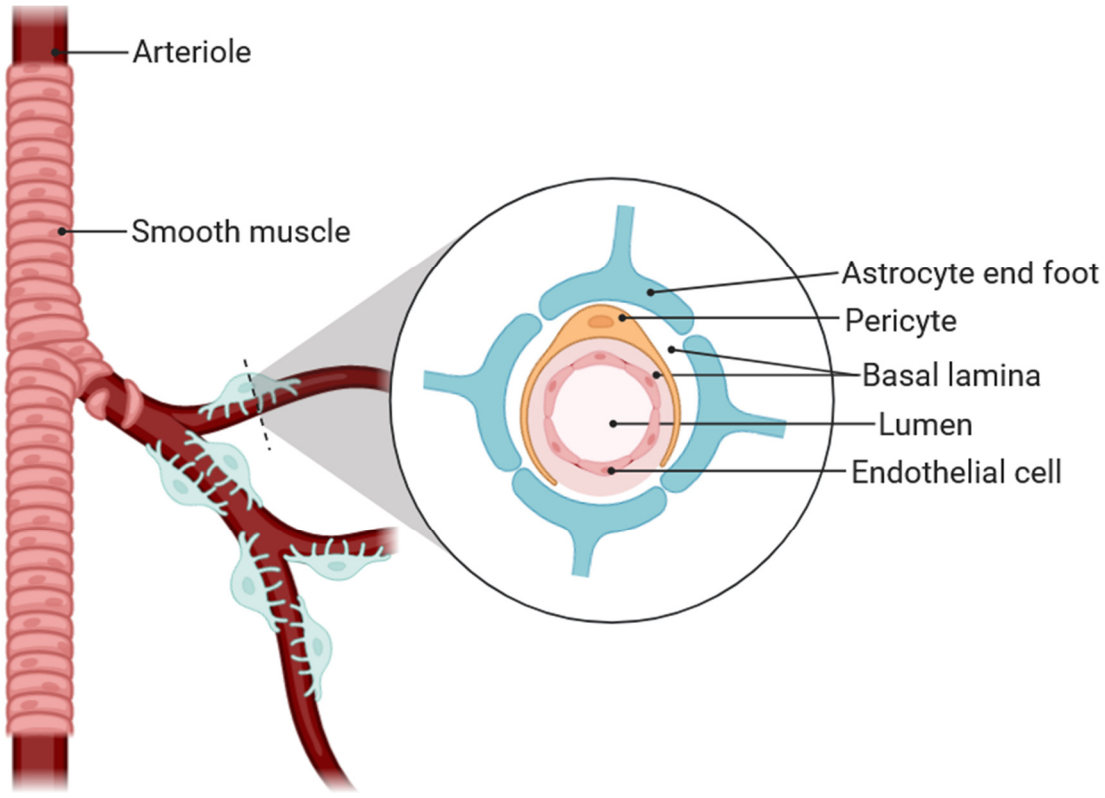

Figure 1. The cellular components of the mature BBB are non-fenestrated endothelial cells, the basement membrane and the surrounding pericytes and astroglial endfeet.

The mature BBB consists of tightly linked non-fenestrated endothelial cells, a basement membrane, pericytes, and astroglial endfeet (Figure 1). These endothelial cells connect to each other via tight junctions (TJs), which consist of proteins like occludin and claudin-5, controlling paracellular permeability. Solute exchange at the BBB is regulated through various transcellular transport systems, including carrier-mediated transport and active efflux, such as that mediated by P-glycoprotein, which is vital for expelling toxic molecules but can be impaired by inflammation and neurodegeneration. The integrity and function of the BBB relies on interactions between endothelial cells, astrocytes, and pericytes. Astrocytes surrounding brain microvessels feature perivascular endfeet characterized with high aquaporin 4 (AQP4) expression. AQP4 enhances water exchange between the brain and blood, which is crucial for CNS homeostasis. However, neuroinflammation can reduce AQP4 expression, impairing BBB function.

Neuroinflammation also triggers a complex response involving pro- and anti-inflammatory cytokines from various cells within the neurovascular unit, including astrocytes and pericytes, which can produce inflammatory cytokines like TNFα and IL-6. These cytokines are involved in diseases like atherosclerosis and their concentrations are elevated in conditions such as Alzheimer's disease and multiple sclerosis, suggesting their role in neurodegeneration. Additionally, microglia in the BBB vicinity can respond to and influence cytokine levels, impacting BBB permeability and cerebral blood flow. On the other hand, cytokines like IL-10 can mitigate some inflammatory responses. There is a complex interplay between various cytokine actions promoting anti- and pro-inflammatory reactions. Consequently, the relationship between vascular inflammation, BBB function, and neurodegeneration remains under-explored.

2. Aim of the study

Our aim was to study BBB related structural and functional changes in APOB-100 transgenic mice that may reveal pathological processes triggered by hypertriglyceridemia, and leading to neurodegeneration. Regarding molecular and cellular mechanisms, we aimed to highlight the effects of IL-6, a pro-inflammatory cytokine which is involved in atherosclerosis, on endothelial and glial cell characteristics, and the possible protective action of an anti-inflammatory cytokine, IL-10. The specific questions of the research projects were the following:

In APOB-100 mice

- are there changes in BBB permeability for small and large marker molecules in the brain cortex and hippocampus?
- does the expression of key BBB genes and cytokines IL-6 and IL-10 change in brain microvessels?
- are there any changes in occludin, claudin-5 and P-gp immunostaining in brain cortex and hippocampus?

Are there differences in the effects of IL-6 and IL-10

- on the growth, viability and density of brain microvascular endothelial cells isolated from WT and APOB-100 mice?
- on the permeability and efflux pump activity of a BBB co-culture model using cells isolated from WT and APOB-100 mice?
- on occludin, claudin-5 and P-gp immunostaining of brain microvascular endothelial cells isolated from WT and APOB-100 mice?
- on the density of cultured primary microglial and astroglial cells isolated from WT and APOB-100 mice?

3. Methods

In the present series of experiments we used a variety of techniques to explore the structure and function of the BBB under hypertriglyceridemic conditions.

In vivo studies

The model of hypertriglyceridemia was validated by monitoring the metabolic status and the serum triglyceride and cholesterol levels in the experimental animals. We examined the functional integrity of the BBB using both small and large molecular markers to evaluate changes in the barrier properties of the BBB, focusing on both paracellular and transcellular pathways. The detection of functional changes was followed by a morphological analysis using immunohistochemistry to visualize the expression of the selected BBB proteins.

In vitro studies

To better understand the cellular mechanisms and interactions affecting the structural integrity and function of the BBB, we cultured brain endothelial cells (BECs), astro- and microglial cells. We observed the kinetics of BEC viability and the effects of cytokine treatments during 24 h.

BBB functional assays included permeability measurements on a double co-culture BBB model, transendothelial electrical resistance (TEER) and P-glycoprotein activity measurements.

Cell cultures of mixed micro- and astroglial cells were also treated with cytokines. Following the functional assays morphological studies were performed to visualize the changes in the expression pattern of proteins playing a key role in barrier function.

Ex vivo studies

To further understand the functional and morphological status of the BBB in hypertriglyceridemia, we isolated brain microvessels. We checked the immunostaining pattern of TJ proteins, P-gp and AQP4 on isolated brain microvessels. Furthermore, changes in the expression levels of several genes coding for various proteins implicated in BBB structure and function were studied using quantitative real time PCR.

Image analysis and Statistical analysis

Image analysis was performed to quantify the obtained immunostaining patterns. The results were statistically analyzed with two-way analysis of variance followed by Bonferroni post hoc test. Data showing no Gaussian distribution were analyzed with Kruskal–Wallis and Dunn's multiple comparison tests.

4. Results

4.1. Chronic hypertriglyceridemia in APOB-100 transgenic mice

The assessment of serum triglyceride levels in APOB-100 transgenic mice at various ages (7, 9, and 12 months) revealed a consistent pattern of elevated triglycerides compared to WT mice. This chronic hypertriglyceridemia highlighted that the expression of the human APOB-100 gene induced lipid metabolism disorders in mice.

4.2. Impairment of BBB integrity: in vivo permeability measurements

In order to investigate the functional integrity of the BBB, we examined the the permeability of two marker molecules which differ in size: the small molecule SF, which provides information on paracellular flux, and the large molecule Evans blue-albumin, a marker of transcellular permeability. We measured a significant increase in BBB permeability ($p < 0.05$) for SF in the hippocampal region of transgenic mice, while alteration in the extravasation of the large serum protein albumin showed a non-significant trend. However, there was no obvious change in the permeability for either of the markers in the cortex of transgenic mice compared to WT littermates.

4.3. Gene expression changes suggesting BBB dysfunction

Quantitative PCR analysis provided insights into the molecular underpinnings of BBB alterations observed in hypertriglyceridemic mice. There was a significant downregulation in the expression of the Meox2 gene, which plays a critical role in maintaining BBB integrity. Similarly, the expression levels of Mfsd2a, a key lipid transporter at the BBB, were substantially reduced. These changes imply a fundamental disruption in BBB structural components and its ability to regulate lipid transport. Conversely, there was an upregulation of the Lox-1 gene, which is implicated in the oxidative stress response to oxidized LDL, suggesting an adaptive response to heightened lipid peroxidation in the transgenic mice. The differential expression of genes related to lipid transport and junction integrity such as Lrp1, Lrp2, and the P-glycoprotein coding genes Abcb1a and Abcb1b further corroborated the findings of a compromised BBB in the context of lipid dysregulation.

4.4. Expression levels of genes coding key cytokines involved in atherosclerotic processes

In order to study the molecular mechanisms leading from hypertriglyceridemia to BBB dysfunction expression levels of genes coding IL-6, a cytokine promoting atherosclerosis, and IL-10, a cytokine that may act as an IL-6 antagonist, were measured in isolated brain microvessels and in brain tissue using qPCR. IL-6 showed a significantly higher expression in cerebral microvessels compared to brain cortex in APOB-100 transgenic mice. IL-10 expression was under the detection limit in each sample.

4.5. Immunohistochemical staining patterns of key BBB proteins

Immunohistochemistry was performed to visualize the localization and expression of crucial BBB proteins, including claudin-5 and occludin, within the hippocampal and cortical regions. Despite the gene expression alterations, the protein expression patterns observed through confocal microscopy did not reveal significant differences in the staining intensity or localization of these proteins between transgenic and WT mice. This observation suggests that post-translational mechanisms or compensatory responses might mitigate the impact of transcriptional changes on protein distribution at the BBB.

4.6. Cell cultures and viability studies

Cell culture studies focused on the viability and functional characteristics of brain endothelial cells derived from both transgenic and WT mice. Our experiments were instrumental in identifying the direct effects of cytokines on cellular health. IL-6 and IL-10 were specifically investigated for their roles in modulating endothelial cell responses under hyperlipidemic conditions. Notably, IL-6 exposure resulted in a decrease in cell viability, which was effectively antagonized by IL-10, highlighting its protective role in inflammatory conditions.

4.7. Brain endothelial cell function assays

Key functions of brain endothelial cells, such as barrier integrity, were evaluated using TEER and permeability tests, along with P-gp activity assays in co-culture BBB models. APOB-100 transgenic endothelial cells co-cultured with APOB-100 transgenic glial cells exhibited reduced TEER values compared to WT cells, with or without cytokine treatments. IL-6 notably decreased TEER in transgenic cells, a process not blocked by IL-10, which alone did not alter TEER values. For WT cells, cytokine treatments significantly lowered TEER.

Permeability to the small molecule SF was higher in APOB-100 cells compared to WT across all groups. IL-6 increased paracellular permeability in WT and even more so in transgenic cells. IL-10 alone did not affect transgenic cell permeability nor mitigate the effects of IL-6. In WT cells, IL-10 alone increased permeability, which was unaffected by IL-6. P-gp activity was reduced in APOB-100 cells, indicated by increased R123 uptake, with no cytokine effect observed in WT cells. In transgenic cells, cytokines increased P-gp activity above basal levels.

4.8. Analysis of endothelial cell morphology

Morphological studies focused on the immunostaining of P-gp and TJ proteins such as claudin-5, occludin, and ZO-1 in primary cultures. P-gp showed decreased immunofluorescence intensity in transgenic versus WT cells under control conditions, with cytokine treatments altering P-gp intensity. IL-6 reduced and IL-10 increased P-gp staining in transgenic cells, with IL-10 also counteracting IL-6 effects (Figure 2.)

Cytokine treatments variably affected the fluorescence intensity of these proteins, with IL-6 and IL-10 influencing their expression in genotype-specific manners. ZO-1 localization was abundant in the cytoplasm post-cytokine treatment, with increased fluorescence in both genotypes (Figure 2).

4.9. Cytokine effects on cultured glial cells

Experiments on mixed astrocyte/microglia cultures from APOB-100 and WT mice showed no change in astrocyte densities. Microglia densities varied with IL-6 increasing and IL-10 decreasing densities in WT cells. The astro/microglia ratio was stable in transgenic cells but decreased in WT cells with IL-6 treatment.

The astroglial endfoot marker AQP4, co-localized with S100b, showed no genotype differences under control conditions. AQP4 immunofluorescence increased in transgenic astrocytes following IL-6 treatment, an effect not observed with combined IL-6 and IL-10 treatment.

4.10. Morphological study of brain microvessels

In isolated brain microvessels, P-gp, claudin-5, occludin, and ZO-1 showed similar patterns and area fractions between genotypes under control conditions. Cytokine treatments altered P-gp and AQP4 expression in WT microvessels, with IL-10 modulating IL-6 effects. Transgenic microvessels displayed no cytokine-induced changes in AQP4 area fraction, even with combined IL-6 and IL-10 treatment (Figure 2).

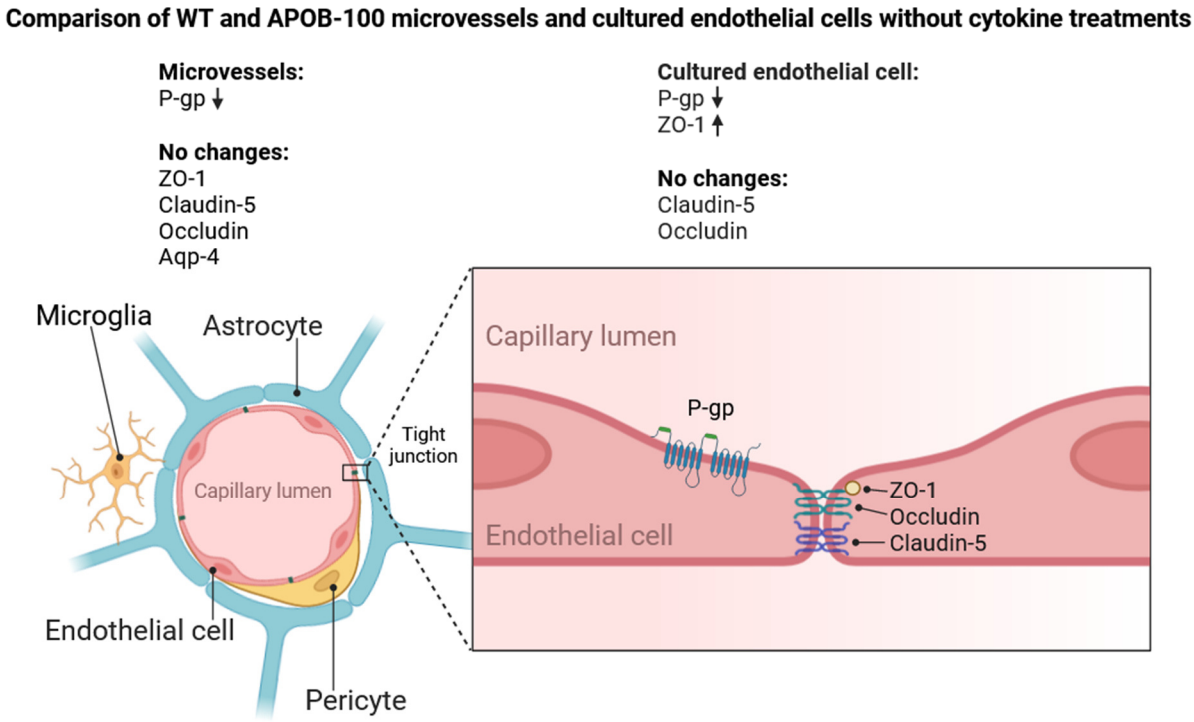

Figure 2. Summary of functional and morphological changes of BBB characteristics comparing WT and APOB-100 models.

5. Discussion

5.1. *In vivo* experiments and chronic hypertriglyceridemia

Our demonstration of chronic hypertriglyceridemia in APOB-100 transgenic mice provides a pivotal foundation for exploring the systemic effects of elevated lipid levels on cerebrovascular integrity. The consistent higher levels of triglycerides in transgenic mice as compared to their WT counterparts not only validate the model but also underscore the role of triglycerides in inducing vascular abnormalities. Previous studies have shown that hypertriglyceridemia can alter BBB properties through mechanisms involving increased oxidative stress and inflammation, which are believed to contribute to endothelial dysfunction commonly observed in metabolic syndromes.

5.2. BBB permeability and integrity

Our findings on BBB permeability reinforce the concept that hypertriglyceridemia compromises the barrier function, primarily affecting the tight junctions that regulate paracellular transport. The differential permeability to small versus large molecules can be

attributed to subtle disruptions in tight junction proteins that are not severe enough to allow large molecules to cross the BBB freely. This selective vulnerability has significant implications for understanding how lipids may exacerbate the progression of neurodegenerative diseases, where the penetration of neurotoxic substances could be facilitated by similar disruptions.

5.3. Molecular insights from gene expression analyses

The gene expression profiles obtained in our study provide molecular insights into the pathophysiological processes underlying BBB dysfunction in hypertriglyceridemia. The downregulation of Meox2 and Mfsd2a is particularly concerning, as these genes are crucial for the structural and functional maintenance of the BBB. Meox2 is known to regulate endothelial cell proliferation and vascular morphogenesis, and its reduced expression could lead to impaired vascular remodelling and stability. Similarly, the decreased expression of Mfsd2a, which is essential for the transport of essential fatty acids across the BBB, suggests that lipid homeostasis within the brain is severely compromised in the hyperlipidemic state.

The increased expression of Lox-1 in response to elevated lipid levels highlights the adaptive changes in endothelial cells facing oxidative stress, suggesting that these cells are in a heightened state of alert, potentially exacerbating inflammatory responses. The alterations in LDL receptor-related proteins (Lrp1 and Lrp2) and P-gp genes further support the notion of a BBB struggling to maintain its protective roles against a backdrop of lipid-induced stress.

5.4. Implications for neurodegenerative diseases

The disruption of BBB integrity has profound implications for the pathogenesis of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. The altered permeability and transport mechanisms at the BBB could facilitate the accumulation of neurotoxic proteins, such as beta-amyloid, in the brain parenchyma. Moreover, the inflammatory milieu characterized by elevated cytokines like IL-6 can exacerbate neurodegeneration by promoting the activation of glial cells and the subsequent release of pro-inflammatory mediators.

5.5. Protective role of IL-10

The cytokine studies revealing the protective effects of IL-10 against IL-6 induced alterations in endothelial cell viability and function are particularly enlightening. IL-10's ability to counteract the effects of IL-6 underscores its potential as a therapeutic agent in conditions where inflammation contributes to disease pathology. By modulating the inflammatory response, IL-10 not only preserves the integrity of endothelial cells but also ensures the proper functioning of the BBB under stress conditions.

6. Summary

Hypertriglyceridemia is a serious risk factor in the development of cardiovascular diseases and closely linked to atherosclerosis related inflammatory processes, BBB dysfunction and neurodegeneration. Using APOB-100 transgenic mice, an animal model of chronic hypertriglyceridemia, our aim was to study cerebrovascular changes, BBB functions and morphology in APOB-100 transgenic mice *in vivo*, *in vitro* and *ex vivo*. Our objective was to determine which BBB characteristics are produced mainly by IL-6, an atherosclerosis promoting cytokine, and whether these actions can be antagonized by IL-10, an anti-inflammatory cytokine.

We monitored the development of chronic hypertriglyceridemia in 7-, 9- and 12-month-old APOB-100 transgenic animals. We found an increased BBB permeability for SF in the hippocampus of APOB-100 transgenic mice which was accompanied by structural changes. In brain microvessels isolated from APOB-100 transgenic animals an increased *Lox-1* and decreased *Meox-2, Mfsd2a, Abcb1a, Lrp2*, *Glut-1* gene expressions were measured using quantitative real-time PCR. IL-6 mRNA levels were higher in isolated brain microvessels than in brain parenchyma of APOB-100 transgenic mice. The decreased P-gp/*Abcb1a* gene expression was reflected at protein level too, as demonstrated by immunohistochemical analysis of transgenic brain sections using confocal microscopy, while in the case of occludin and claudin-5 TJ proteins, no changes in immunolabeling pattern was observed.

In our next series of experiments, we demonstrated functional and morphological differences between WT and APOB-100 brain endothelial cells under control conditions. Functional characteristics, such as TEER, SF permeability and P-gp activity were sensitive to both IL-6 and IL-10 cytokine treatments, but no antagonistic effect was observed. In

contrast, a decrease in brain endothelial cell density and P-gp immunofluorescence intensity detected in both genotypes following IL-6 treatment was antagonized by IL-10. Other BBB features sensitive to IL-6 in APOB-100 brain endothelial cells included a decrease in claudin-5 and an increase in AQP4 fluorescence intensity, and a decrease in astro-/microglia ratio in WT glia cultures. These IL-6 induced changes were also antagonized by IL-10. Isolated brain microvessels in general, and APOB-100 microvessels in particular, were less reactive to cytokine treatments than cell cultures. In this *ex vivo* system IL-6 resulted in a decrease in P-gp, ZO-1 and AQP4 immunostained area fractions in WT microvessels. The decrease in AQP4 immunolabeled area fraction was antagonized by IL-10. Following treatment with the anti-inflammatory cytokine IL-10 a decrease in P-gp and ZO-1 immunolabeled area fractions was seen, and no antagonistic effect was observed between IL-6 and IL-10 action regarding P-gp and ZO-1 changes in WT microvessels. Our present results identify BBB characteristics sensitive to either IL-6 or IL-10 actions, and demonstrate for the first time that IL-10 can prevent, at least in part, IL-6 induced BBB impairments.

7. Conclusions

Based on our results we conclude that in chronic hypertriglyceridemic APOB-100 transgenic mice both functional and morphological cerebrovascular pathology can be observed. Therefore, this animal model could be a useful tool to study the link between cerebrovascular damage and neurodegenerative diseases of vascular origin, which is fundamental for the development of efficient therapies.

8. Acknowledgments

I would like to express my gratitude to my supervisors, Prof. Mária Deli and Dr. Zsófia Hoyk, for their support and help they gave during my doctoral studies. The completion of the experiments and preparation of the present dissertation would not have been possible without their guidance.

I am grateful for the kind support and scientific guidance to Dr. Fruzsina Walter from the Biological Barriers Research Group.

I would like to say thank you to Prof. Miklós Sántha, Dr. Melinda Tóth, Dr. Brigitta Dukay from the Institute of Biochemistry, BRC, for their help and collaboration.

I would like to gratefully thank all members of the Biological Barriers Research Group for working together and having their support. I am especially grateful to Dr. András Harazin, Dr. Lilla Barna, Dr. Ana Raquel Santa Maria, Dr. Réka Molnár and Judit Vigh for the long working hours we spent together.

I would like to mention Balázs Hallgas, Eszter Lomen, Éva Kárpáti, members of Navolab Diagnostics team. I owe them with my gratitude to have their support to accomplish my studies.

I would like to thank all the people who helped and supported me during my work, they all contributed to make it happen.

My special thanks and gratitude go for my family and closest friends. They have always had my back.

I acknowledge my gratitude to the Theoretical Medicine Doctoral School for permitting me to participate in their doctoral program and their support.