Hyperlipidemia induced neurodegenerative and blood-brain barrier changes in ApoB-100 transgenic mice

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Ph. D. thesis summary

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Introduction

In the last few decades several clinical studies aimed to understand the connection between hyperlipidemia (mainly hypercholesterolemia) and age-related cognitive disorders (dementia). In the new neurovascular concept, emphasis was laid on the vascular aspect in the pathomechanism of dementias, therefore investigations on the cerebrovascular alterations came to the front. Cognitive decline is frequently accompanied by dyslipidemia and atherosclerosis in elderly people. The underlying mechanisms of vascular dementia are high LDL-cholesterol level, stroke, trauma, encephalitis, hypertonia, diabetes, and obesity. The high level of low density lipoproteins (LDL) leads to the development of atherosclerosis and cardiac failure. ApoB-100 is the major protein component of VLDL and LDL particles

We have been investigating the possible link between hyperlipidemia and neurodegeneration in hApoB-100 transgenic mice, which strain fed on lipid-rich diet is a validated model of atherosclerosis. Previously, this mouse strain was generated and characterized in our laboratory. We have shown that upon normal diet these mice were hypertriglyceridemic, while on cholesterol-rich diet they had elevated serum cholesterol level, which led to increased oxidative stress and lipid peroxidation. We have also found an increase in the level of membrane-bound APP in the cortex of transgenics. Transgenic mice fed on normal chow have high triglyceride level and they show extensive cortical and hippocampal apoptosis, neurodegeneration eventually leading to the enlargement of the brain ventricles. Under ischemic conditions the density of the cortical capillary network was significantly reduced and as a compensatory mechanism, the diameter of the capillaries were increased.

Aims of the study

We have been investigating the link between hiperlipidemia and neurodegeneration with the help of our hypertriglyceridemic human ApoB-100 overexpressing transgenic mice. We hypothesize that chronic hiperlipidemia not only affects the cardiovascular, but the cerebrovascular system as well, it might induce damages in the cerebral vessels. The impairment of the endothelium is accompanied by brain hypoperfusion and ischemia, which triggers oxidative stress, eventulally leading to blood-brain barrier breakdown. These changes may initiate neurodegenerative processes, which are characterized by synaptic and neuronal dysfunction, ultimately leading to neuronal apoptosis.

The following questions were raised during our experimental work:

- What is the effect of systemic chronic hyperlipidemia on cerebral lipid metabolism?
- How the functional integrity of the blood-brain barrier is influenced by hyperlipidemia?
- Which are the main features of hyperlipidemia induced neurodegenerative changes?
- How hyperlipidemia induced endothelial dysfunction can be modelled in vitro with the help of isolated primary cells of the blood-brain barrier?

Methods

- Colorimetric serum triglyceride measurement
- Lipid stainings on brain cryosections: Nile red and Filipin stainings
- Histological examinations on brain cryosections: Immunohistochemistry, Congo red staining, Fluoro-Jade C staining, Golgi-Cox impregnation
- Semi-quantitative and quantitative western blottings on cortical samples
- Electrophysiological recordings (PPF, LTP) on hippocampal brain slices
- *In vivo* examination of blood-brain barrier integrity (parenchymal Sodium-fluorescein and Evans-blue permeability)
- Gene expression studies on isolated cortical microcapillaries
- Isolation and cultivation of primary capillary endothelial cells, pericytes and astrocytes
- Immunocytochemistry on primary endothelial cells, astrocytes and pericytes
- *In vitro* real-time cell analyzing (using the xCELLigence system), and cytotoxicity test (LDH test) on isolated primary cells of the blood-brain barrier
- *In vitro* ROS measurements on isolated primary cells of the blood-brain barrier

Part of the experiments were carried out in collaboration with other laboratories. Investigations on the morphological changes of neurodegeneration and electrophysiological recordings were carried out with

the help of Botond Penke PhD, DSc, Viktor Szegedi PhD and coworkers of the Neurodegeneration Research Laboratory (University of Szeged, Faculty of Medicine, Department of Medical Chemistry). Investigations on the blood-brain barrier integrity and isolated primary cells of the blood-brain barrier were performed with the help of Maria Deli MD, PhD, DSc and coworkers of the Biological Barriers Research Group. (Hungarian Academy of Sciences, Biological Research Centre, Institute of Biophysics).

Results

First, the effect of chronic hypertriglyceridemia on cerebral lipid metabolism was investigated. We found that the cerebral level of ApoE and LDLr was significantly increased, whereas the cerebral level of ApoA-I was decreased in transgenic mice compared to wild type littermates. The high amount of ApoE and LDLr possibly refers to increased cellular cholesterol metabolism, while the reduction of ApoA-I can contribute to reduced tolerance in inflammation and oxidative stress caused by ROS on brain capillary enothelial cells. The high level of serum triglycerides led to the formation of intracellular lipid droplets in the brain parenchyma of transgenic animals. Although, we found that the amount of cortical APP was increased, no amyloid plaques were detected in the brain tissue of heterozygote transgenic animals. This can be the consequence of the elevated ApoE level, which is responsible for facilitating the clearance of Aβ peptide.

We have also demonstrated that the Tau protein is hyperphosphorylated at several sites (TaupSer$^{262,\ 199/202,\ 396,\ 404}$) in the cortex

of transgenic animals compared to wild type, which histologically leads to the formation of neurofibrillary tangles ($TaupSer^{404}$). The hyperphosphorylation of Tau disorganizes the microtubule network, impairs the axonal transport, synaptic transmission and eventually leads to neuronal cell death. These neurodegenerative changes manifest in extensive dendritic spine-density reduction, cortical and hippocampal apoptosis of neuronal cells. In parallel with these morphological changes we revealed impaired learning, memory (decreased LTP amplitude), and short-term synaptic plasticity (reduced PPF ratios) and simultaneously pronounced reduction in PKC-γ protein level.

Due to the evident morphological and functional alterations detected in transgenic brains we raised the question how chronic hyperlipidemia might affect permeability of the blood-brain barrier (BBB). We found, that hyperlipidemia notably, but not significantly, increased the paracellular permeability of the BBB in transgenic mice. We have also observed that lipid diet had pronounced effect on transcellular permeability, as it significantly increased vascular permeability for large molecules in all investigated brain area in both transgenic and wild type animals.

We found a high expression level of the ApoB-100 and LOX1 in isolated cortical microcapillaries and detected strong ApoB immunoreactivity in primary endothelial cells, pericytes and astrocytes derived from transgenic animals. Löffler et al. have shown perivascular accumulation of ApoB in the brain of transgenic mice referring to vascular lesions and BBB damage. However, further investigations are needed to reveal the harmful effect of the accumulated ApoB protein in the brain. Using immunostaining we showed the presence of ApoB-100 protein in wild type endothelial cells, astrocytes

and pericytes. This was a novel observation and was not published earlier. Under normal physiological conditions ApoB-100 presumably helps in better utilization of lipids on the cellular level. Similar effect was observed during cultivation of primary capillary endothelial cells and astrocytes. Cells originated from transgenic animals grew significantly better and faster compared to wild type cells using the same cultivation conditions. The elevated expression level of LOX1 in the microvessels may refer to increased oxidative damage and cerebrovascular lipid peroxidation. In connection with this finding we monitored the sensitivity of primary cells subjected to oxLDL treatment. We have shown that primary cell types have different vulnerability to oxLDL. A dose-dependent reduction in cell viability was noticed in all oxLDL treated cell types. Astrocytes and endothelial cells were the most, and pericytes the less sensitive to the treatment. The viability results were confirmed by LDH test. We observed that wild type astrocytes and endothelial cells show significantly higher degree of membrane damage and reduction in viability than transgenic cells. In contrast, pericytes were less vulnerable, they showed a mild decrease in viability and strong membrane impairment. To elucidate the reason for this phenomenon we measured the ROS production of the cells during oxLDL treatment. We found that in transgenic endothelial cells and pericytes the basic ROS production is significantly higher than in wild type cells, which is further enhanced by the oxLDL treatment in transgenic cells. Regarding astrocytes the opposite was true: wild type cells produced significantly more ROS than transgenic cells. Each of our results are consistent with data found in the literature, but further investigations are needed to elucidate the exact mechanism of endothelial dysfunction caused by oxLDL.

Major results

- We have demonstrated that due to the significantly elevated serum triglyceride level triglyceride-rich intracellular lipid droplets could be detected in the brain of transgenic mice .

- The level of cerebral ApoB, ApoE and LDLr is significantly higher in the cortical region of transgenic mice compared to the wild type. In contrast, the cerebral level of ApoA-I is significantly lower in transgenics.

- Although the expression level of APP found in the cortical region of transgenic mice was markedly high, no Aβ plaques could be detected in brain tissue of heterozygote transgenic mice.

- We have shown that hyperphosphorylation of Tau protein occurs very early in transgenics compared to wild type mice. As a consequence of hyperphosphorylation neurofibrillary tangles could be visualised in the brain tissue of transgenics.

- Extensive neuronal apoptosis can be detected in the cortical and hippocampal regions of transgenic brains. In addition, the number and length of dendritic spines are also reduced in the hippocampal area of transgenics compared to wild type mice.

- After high fat diet the permeability of blood-brain barrier for large molecular weight compounds were found to be significantly increased in both transgenic and wild type mice. .

- We have shown that ApoB-100 protein can be found in the isolated primary cells (endothelial cells, astrocytes, pericytes) of the blood-brain barrier of wild type mice.

- The sensitivity of isolated primary cells to oxLDL treatment differ from each other. Endothelial cells and astrocytes seem to be more vulnerable compared to pericytes in both genotype.

- Endothelial cells and pericytes derived from transgenic animals produce significantly more ROS than cells isolated from wild type mice.

Conclusions and final remarks

Our hypothesis was that the cerebrovascular system was strongly affected by chronic hyperlipidemia in hApoB-100 transgenic mice. As a consequence of hyperlipidemia atherosclerotic lesions were developed in the cerebrovascular system, which led to brain hypoperfusion, oxidative stress and inflammation. The oxidized lipids, especially oxisterols initiated endothelial dysfunction and disruption of the BBB. The damage of the BBB and the existing cerebral ischemia induced neurodegenerative processes in the brain. Our results showed that the hApoB-100 mouse strain is a versatile model of hyperlipidemia induced neurofibrillary degeneration.

List of publications

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Lénárt N, Szegedi V, Juhász G, Kasztner A, Horváth J, Bereczki E, Tóth ME, Penke B, Sántha M: **Increased Tau Phosphorylation and Impaired Presynaptic Function in Hypertriglyceridemic ApoB-100 Transgenic Mice** PLOS ONE 7:(9) Paper e46007. 12 p. (2012) IF: 3.730

Tóth ME, Szegedi V, Varga E, Juhász G, Horváth J, Borbély E, Csibrány B, Alföldi R, Lénárt N, Penke B, Sántha M: **Overexpression of Hsp27 ameliorates symptoms of Alzheimer's disease in APP/PS1 mice** CELL STRESS & CHAPERONES 18:(6) pp. 759-771. (2013) IF: 2.537

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Zádor F, Lénárt N, Csibrány B, Sántha M, Molnár M, Tuka B, Vécsei L, Marton A, Vízler Cs, Oláh M, Borsodi A, Benyhe S, Páldy E: **Low dosage of rimonabant leads to anxiolytic like behavior with a cannabinoid receptor independent manner via inhibiting expression levels and G-protein activity of kappa opioid receptors.**Neuropharmacology 89, 298-307. (2015)

Dénes Á, Coutts G, Lénárt N, Pelegrin P, Skinner J, Rothwell N, Allan MS, Brough D: **AIM2, NLRC4 and ASC inflammasomes contribute to acute brain injury** (2014) manuscript in preparation

poster presentations:

Lénárt N, Szegedi V, Juhász G, Kasztner A, Tóth EM, Penke B, Sántha M **Chronic hypertriglyceridemia induces early tau hyperhosphorylation and impaired long-term potentiation in apoB-100 transgenic mice** 2012. Straub-Days, Szeged

Lénárt N, Bernát G, Scheich H, Sánhta M. **Hyperlipidemia induced neurodegeneration in apoB-100 transgenic mice** 2012. CEELA, Budapest

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