Brain sterol metabolism: modulating Alzheimer’s disease

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Summary / Samenvatting
**Summary**

Alzheimer’s disease (AD) is the most common form of elderly dementia, leading to a high burden on caregivers and costs to society. Research on the causality and treatment of AD is therefore of high social and economic importance. Based on immunohistological hallmarks featuring *post mortem* diagnosis of AD, several hypotheses concerning the causality of late onset AD were postulated. The rationale in this thesis is based on the amyloid-β (Aβ) hypothesis. The close association between the development and progression of Aβ dependent AD pathology and alterations in the cholesterol metabolism are extensively described in *chapter I*. The peripheral cholesterol metabolism is clearly separated from the cerebral cholesterol metabolism. Whereas the influence of plasma cholesterol concentrations is rather indirect, alterations in the brain cholesterol metabolism are described to be directly involved in the development and progression of AD. A detailed overview of the most important cerebral cholesterol regulatory mechanisms is provided in the general introduction, along with their relation to AD. In addition, the exogenous cholesterol analogues, plant sterols, and the potential consequences on their cerebral accumulation are introduced.

In *chapter II* of the thesis, we questioned whether alterations in the cerebral cholesterol metabolism are cause or consequence of the amyloid pathology in the brain. We applied a unique AD mouse model, possessing a more physiological expression pattern of the involved APP gene, namely the APPSLxPS1mut mouse. Brains and serum of APPSLxPS1mut mice and control littermates were examined at different ages: at the pre-plaque stage (9 months) versus the advanced pathology stage (21 months). Different parameters in the brain cholesterol metabolism were measured and compared: cholesterol, its precursors and metabolites, and genes involved in cholesterol synthesis, transport and metabolizing. We found age-related alterations in their brain sterol profiles and in the expression of cholesterol metabolism-related genes in comparison with wild-type mice. Concentrations of 24(S)-OHcholesterol and desmosterol were only modestly affected at 9 months, whereas they were significantly increased in 21 months old APPSLxPS1mut mice in comparison to their healthy littermates. In parallel, the liver x receptor (LXR) responsive genes, Apoc1 and Abca1 displayed an increase in mRNA expression pattern. We therefore concluded that the alterations in the cerebral cholesterol metabolism are rather a consequence of the AD pathology in the brains of APPSLxPS1mut mice. We suggested that the increased cerebral cholesterol
turnover attempts to rescue and to cope with the cerebral AD pathology. Yet, we did not exclude a causative influence of an altered cerebral cholesterol metabolism in the development of Alzheimer’s disease pathology.

In line with the outcome of the experiments obtained in chapter II, we fed aged APPSLxPS1mut mice a synthetic LXR agonist (T0901317) over 10 weeks in the terminal phase of the disease pathology (chapter III). We aimed to reverse/prevent Aβ related AD pathology and the parallel cognitive deficits. Upon this long term LXR activation, cognitive performances were significantly improved compared to the untreated APPSLxPS1mut mice. Surprisingly, in contrast to our hypothesis, Aβ deposition was not reduced upon LXR activation. Our data suggest that rather an enhanced cholesterol flux is underlying the beneficial effects of T0901317. Cerebral cholesterol turnover may therefore be a suitable target in the treatment and/or retardation of neurodegenerative diseases, including those other than AD.

Several plant sterols, exclusively derivable form dietary origin are classified as exogenous, naturally occurring LXR agonists. Chapter IV deals with the consequences of the recently described accumulation of plant sterol within mice brains. In this chapter, we questioned the fate and the reversibility of cerebral plant sterol accumulation in mice brains. In addition, we investigated the capacity of the most prevalent plant sterols (sitosterol, campesterol and stigmasterol) to modulate Aβ production in an in vitro model for AD. We demonstrated that 6 weeks of dietary enrichment with plant sterols resulted in an irreversible accumulation of plant sterols in the brains of normal mice, despite a drastic reduction of circulating plant sterol level upon 6 subsequent months. Moreover we found that campesterol is more efficiently transferred across an in vitro blood-brain barrier (BBB) model compared to sitosterol. Behind the BBB, plant sterols are incorporated within the cholesterol rich lipid raft membrane domains of brain cells. A neuronal cell line stably over-expressing the amyloid precursor protein (APP), displayed a reduced processing of APP upon sitosterol administration. Together, these data pointed to the potential therapeutical implications for plant sterol enriched food spreads in the treatment and prevention of AD.

In chapter V, we investigated the functional consequences of cerebral plant sterol accumulation in mice. We investigated cognition, anxiety and motor skills in ATP binding-cassette transporter (Abc)g5 knockout mice, bearing strongly increased brain plant sterol concentrations. Except for an increased swimming speed in the Morris water escape maze, none of the above mentioned brain functions were influenced by the increased cerebral accumulation of plant sterols. However,
whether plant sterols can modify cognitive decline in a model for AD remains to be elucidated.

In chapter VI, we hypothesized, based on the solely exogenous nature of plant sterols in the CSF and the impaired choroid plexus function in AD, that plant sterol concentrations in CSF are reduced in AD pathology and therefore can be added to the relevant spectrum of biomarkers for AD. We found that, despite comparable plasma plant sterol concentrations, absolute sitosterol and brassicasterol concentrations were significantly reduced in cerebrospinal fluid (CSF) of AD patients. After correction for CSF cholesterol concentrations, only brassicasterol was found to be a relevant biomarker for AD.

Together, our findings provide more insight into the role of cerebral cholesterol turnover in AD. The main findings are described and reflected against the up to date literature in the general discussion (chapter VII).
Samenvatting

Alzheimer’s disease (AD), the most common form of dementia in older people, leads to a high burden on caregivers and to high costs for society. Research into the causes and treatment of AD is therefore of great social and economic importance. Based on immunohistological characteristics that mark the post-mortem diagnosis of AD, several hypotheses about the causality of “late onset” AD have been proposed. The hypotheses presented in this dissertation are based on the amyloid-β (Aβ) hypothesis. The close relationship between the development and progression of Aβ-dependent pathology in AD and the associated changes in the cholesterol metabolism are described in Chapter I. The peripheral cholesterol homeostasis is separated from the cerebral cholesterol metabolism. Changes in plasma cholesterol concentrations are indirectly associated with the development of AD. However, changes in the cholesterol metabolism in the brain is described as directly involved in the development and progression of AD. In the general introduction, a detailed overview of the most important cholesterol regulation mechanisms in the brain in relation to AD is given. Furthermore, the exogenous cholesterol analogs, namely plant sterols, and the possible consequences of their accumulation in the brain are described.

In Chapter II of this dissertation, we asked whether changes in the cerebral cholesterol metabolism are rather a cause or a consequence of the amyloid pathology in the brain. We used a unique AD mouse model, which shows a more physiological expression pattern of the involved APP-gen, namely the APPSLxPS1mut mouse. Brain and serum of APPSLxPS1mut and control mice were studied at different ages: (1) at the pre-plaque stage (9 months) and (2) at the late stage of pathology (21 months). Various parameters of the cholesterol metabolism in the brain were measured and compared: cholesterol precursors, cholesterol itself, cholesterol metabolites and genes involved in cholesterol synthesis, transport and metabolism. In comparison with wild-type mice, we observed age-related changes in cholesterol metabolism in the brain and in the expression of cholesterol metabolism-related genes. Concentrations of 24(S)-OHcholesterol and desmosterol were influenced to a moderate extent at 9 months, while they were significantly increased at 21 months, while their significant increase was observed in 21-month-old mice.
APPSSLxPS1mut muizen in vergelijking met hun niet-AD nestgenoten. Parallel hieraan was er een verhoogde transcriptie van de liver X receptor (LXR) responsieve genen, Apoc1 en Abca1. Daarom concludeerden we dat de veranderingen in het cerebrale cholesterol metabolisme een gevolg konden zijn van de AD-pathologie in de hersenen van APPSSLxPS1mut muizen. We suggereerden daarom dat de verhoogde turnover van het cerebrale cholesterol dient om de hersenen te redden van de cerebrale amyloid pathologie in AD. Desondanks sluiten we niet uit dat veranderingen in het cholesterol metabolisme in de hersenen de ontwikkeling van AD mede kan veroorzaken.

Gebaseerd op de resultaten verkregen in hoofdstuk II, besloten we oude APPSSLxPS1mut muizen 10 weken lang met een synthetische LXR agonist (T0901317) in de terminale fase van hun AD pathologie te behandelen (hoofdstuk III). Ons doel was de Aβ gerelateerde pathologie en de daaraan verbonden cognitieve achteruitgang om te keren of te voorkomen. Volgend op de langedurige LXR activatie werden de cognitieve prestaties aanzienlijk verbeterd in vergelijking met de onbehandelde APPSSLxPS1 muizen. In tegenstelling tot onze hypothese was de Aβ depositie niet verminderd na LXR activering. Onze gegevens suggereren dat een verbeterde cholesterol flux meer waarschijnlijk ten grondslag ligt aan de gunstige effecten van T0901317. Cerebrale cholesterol turnover kan dus een geschikt doelwit voor de behandeling en vertraging van neurodegeneratieve ziekten in het algemeen worden.

Verschillende plantensterolen zijn geclassificeerd als exogene, natuurlijk voorkomende LXR agonisten. Hoofdstuk IV behandelt de gevolgen van de onlangs beschreven accumulatie van plantensterolen in muizen hersenen. In dit hoofdstuk, stelden we het lot en de omkeerbaarheid van cerebrale plantensterolen accumulatie in muizen hersenen in vraag. Daarnaast onderzochten we de mogelijkheid van de meest voorkomende plant sterolen (sitosterol, campesterol en stigmasterol) om Aβ productie te moduleren in een in vitro model voor AD. We hebben aangetoond dat 6 weken van dieet verrijking met plantensterolen resulteerde in een onomkeerbare ophoping van plantensterolen in de hersenen van normale muizen, ondanks een verdere afname van serum plantensterol concentraties bekomen door een plantenstanolen verrijkt dieet gedurende 6 daarop volgende maanden. Bovendien vonden we dat campesterol in vergelijking met sitosterol efficiënter over de bloed-hersenbarrière (BHB) wordt getransporteerd. Achter de BBB worden plantensterolen opgenomen in de cholesterolrijke” lipid raft” membraan domeinen van hersencellen. Een neuronale cellijn die het amyloid precursor proteïne (APP) stabiel tot overexpressie brengt, toont een verminderde productie van Aβ na
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toediening van sitosterol. Samen duiden deze gegevens op potentiële therapeutische implicaties voor plantensterolen verrijkte voedingsmiddelen in de preventie en behandeling van AD.

In hoofdstuk V, onderzochten we de functionele gevolgen van de accumulatie van plantensterolen in muizen hersenen. We onderzochten cognitie, angst en motorische vaardigheden in de ATP-binding cassette transporter (Abc)g5 knock-out muizen. Abcg5 knock-out muizen vertonen sterk verhoogde concentraties aan plantensterolen in het plasma en brein. Uitgezonderd de hogere zwemsnelheid in de Morris water escape maze, werden geen van de hierboven genoemde functies van de hersenen beïnvloed door de toegenomen cerebrale accumulatie van plantensterolen. Echter, of plantensterolen de cognitieve achteruitgang in een model voor AD kan voorkomen of terugdringen moet nog worden opgehelderd.

Gebaseerd op de uitsluitend exogene aard van plantensterolen in de hersenen en de verminderde plexus choroideus functie in AD, onderzochten wij in hoofdstuk VI de hypothese dat plantensterolen concentraties in liquor van AD patiënten lager liggen dan in controle patiënten en daardoor dus mogelijke biomarkers voor AD zijn. We vonden dat, ondanks vergelijkbare plasma concentraties aan plantensterolen, absolute sitosterol en brassicasterol concentraties aanzienlijk lager waren in de liquor van AD patiënten. Na correctie voor liquor cholesterol concentraties, vonden wij dat brassicasterol een een relevante biomarker voor AD is.

Tot slot bieden onze bevindingen meer inzicht in de rol van de cerebrale cholesterol turnover in de ontwikkeling en preventie van AD. De belangrijkste bevindingen worden beschreven en gereflecteerd tegen de up to date literatuur in de algemene discussie (hoofdstuk VII).