Alzheimer's disease prevention – The emerging role of lipids and diet

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Abstract: *Although Alzheimer's disease (AD) causes massive and irreversible neurodegeneration, prevention and curing early stages of the disease appears to represent a realistic goal to be achieved in future. In fact, one of the very first effective treatments available could be derived from ordinary food sources.*
Overproduction of the amyloidogenic peptide Aβ42 causes AD. Thus far two physiological regulatory cycles were identified in which Aβ peptides play a major role. These regulatory cycles are involved in cholesterol and sphingolipid homeostasis. Moreover, Aβ production is under physiological conditions tightly regulated and its production rate is highly sensitive to alterations of the cellular membrane composition. Several lipids, sterols and fatty acids have thus far been identified to affect Aβ production. Most knowledge thus far has been gathered about those lipids which are themselves are target of Aβ mediated lipid homeostasis, cholesterol and sphingomyelin. E.g. cholesterol strongly increases Aβ production and cholesterol lowering with statins is a matter of intense clinical research not only for cardiovascular disease preventions but now also for AD therapy. Special interest received n-3 polyunsaturated fatty acids, especially DHA, because of their Aβ lowering effect in combination with favorable pharmacokinetics and neuroprotective properties.

Key words: *Alzheimer's disease, DHA, diet, neurodegeneration, prevention*

Introduction

When Alois Alzheimer a century ago identified amyloid plaques as the hallmark and molecular manifestation of dementia in the elderly, he set the stage for what has now become a major scientific effort – treating and preventing Alzheimer's disease (AD).
Dementia and lipid physiology, especially concerning cholesterol, have recently been discovered to be very closely linked. This linkage has been observed on all levels of research. Until recently little indication existed for a link between AD, Amyloid Precursor Protein (APP) processing and cholesterol homeostasis. However, this picture has changed dramatically over the last years. First indications that lipids may play an important role in APP processing and Aβ production are given by the finding that all proteins involved in APP processing are integral membrane proteins. Taking into consideration that the Aβ producing cleavage by the γ-secretase takes place in the middle of the membrane it is reasonable to assume that the lipid environment of the cleavage enzymes influences Aβ production and hence AD pathogenesis [1]. Moreover, cellular and biochemical studies show that APP processing and the proteases involved are sensitive to cholesterol and cholesterol trafficking [2-6]. *In vivo* studies revealed that cholesterol feeding increases Aβ42 production and amyloid burden whereas lowering cholesterol by medication (e.g. statins) decreases Aβ production and amyloid burden [3, 7, 8]. Accordingly statin treatment is associated with a reduced AD risk in some epidemiological studies [9-11]. First preliminary clinical trials have led to mixed results. High statin dosage reduced cerebral Aβ levels and disease progression in mild AD [8]. When treatment duration was doubled beneficial effects were observed in mild and moderate AD [12]. However, low statin dosage treatment for up to twelve months did not show, aside from some potential secretase inhibition, any clinical benefit [13, 14]. It is now clear that the Aβ generating machinery is an integral part of the body's lipid homeostasis regulating system which causes it to respond very sensitively to changes in lipid levels [15]. Moreover, it is becoming ever more evident, that there are several more routes through which lipids can have either a beneficial or a detrimental impact on the brain. Especially the n3-fatty acids docosahexaenic acid (DHA) and eicosapentaenoic acid (EPA) represent promising candidate and a first clinical trial ended with positive results.

Disease etiology

Alzheimer's disease is a progressive neurodegeneration typically affecting the elderly. There is currently no cure or prevention available. Treatment is limited to symptomatic interventions which offer some relief to patients and care givers for a limited time. While there has been major progress in AD research, non-symptomatic treatments are still in an experimental stage [16]. A conglomerate of individual risk factors including genetic and environmental factors causes the vast majority of AD cases. AD can be caused by auto-somal dominant mutations too. While these familial AD cases occur only very rarely the identification of the mutated genes has let to the decipherment of important molecular mechanism leading to AD. From this it has been concluded that enhanced production of Amyloid beta (Aβ) peptides cause AD. Especially overproduction of the long Aβ42 peptide causes early onset Alzheimer's disease. Whereas in disease extreme amounts of these peptides accumulate in the brain, their levels remain low outside of the brain and in absence of AD. Because of the accumulation of Aβ42 in all AD cases it is assumed that such Aβ42 overproduction is likewise involved in the pathogenesis of sporadic AD as well. Aβ is a physiological cleavage product from the APP, a protein of uncertain function which is ubiquitously expressed, but expression is especially high in neurons. Aβ release is a two step procedure. Cleavage is initiated by BACE 1, which is followed by γ-secretase cleavage. Both proteases are membrane bound, but only the latter one cleaves intramembranously. The proteolytic cascade resulting in Aβ release is, that of a regulated intramembrane proteolysis (RIP). RIP processing had first been recognized in cholesterol *de novo* synthesis up-regulation. Very few cellular processes are determined by a RIP mechanism and once this coincidence was noted it was suggested that there this mechanistic similarity might also indicate functional overlaps [17, 18].

Indeed, Aβ production is under physiological conditions tightly regulated and its production

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rate is highly sensitive to alterations of the cellular membrane composition.

Mechanistic lipid link

Vascular factors and factors related to diet, including blood lipid levels and adiposity, have been linked with an increased risk of dementia and AD. In addition, the Apolipoprotein Eε4 allele (APOE), a protein involved in lipid metabolism, is the most frequent genetic susceptibility factor for dementia. These studies have provided the basis for first reports and replication studies on relationships between overweight and obesity and AD [19, 20], high adiposity and cerebro-vascular diseases [21, 22], an obesity- and sex hormone-related marker and blood brain barrier integrity, high blood cholesterol in midlife and subsequent AD [20, 23], and low blood cholesterol and AD in late life [24] and high blood pressure both at midlife and late-life and AD [25, 26]. It was also shown that a combination of midlife hypertension, obesity, and hypercholesterolemia increases the risk of dementia 6-fold [27]. Insights into the molecular mechanisms governing the molecular link between lipid homeostasis and AD were gained when APP γ-secretase knock-out animals and cells were investigated. The γ-secretase is a multi-metric protease complex, which does the final Aβ releasing intramembrane scission in APP. Moreover, it is this very proteolytic event which determines whether the potentially neurodegeneration causing Aβ42 or the two amino acids shorter Aβ40 is produced. Aβ40 overproduction might increase the risk for vascular dementia, but is not known to represent a risk for AD. Typically γ-secretase produces 10-times more Aβ40 than Aβ42. Mutations in the active center of γ-secretase, which is formed by one of the two presenilin genes (PS1 and PS2) shift the balance towards Aβ42. Indeed there is a direct correlation between the age of disease onset and the overproduction rate of Aβ42 [28]. The relevance of Aβ42 overproduction for AD is further confirmed by familial AD mutations in the APP gene. These mutations predominantly increase Aβ42 production too. Absence of both PS genes abolishes the cellular γ-secretase activity entirely. The PS knock-out causes early embryonic lethality, which presumably is not due to altered APP processing but to defective processing of other γ-secretase substrates. However, cells and conditional knock-out animals are viable and can be studied. PS knock-out cells show a peculiar lipid phenotype which resembles a defective sterol regulatory binding protein defect, because cholesterol *de novo* synthesis is strongly increased in PS1/ PS2 double knock-out cells and tissue of conditional knock-out animals. However, not only cholesterol levels are affected, but sphingolipids are equally increased, extending the impact of these mutations. Curiously, PS mutations which cause early onset familial AD differ somewhat in their effect. There cholesterol levels are still notably elevated, as compared to the wild type situation, but sphingolipids are not increased but rather than that decreased [15]. The answer to this phenomenon became clear when wild type cells were treated with γ-secretase inhibitors. Even in the presence of γ-secretase the inhibition of the proteolytic activity was sufficient to mimic the PS knock-out phenotype with increased cholesterol and sphingomyelin levels. Therefore γ-secretase has to influence lipid homeostasis via one of its proteolytic substrates. The analysis of APP knock-out cells and animals then revealed that this substrate is APP, the Aβ peptide precursor. Moreover, lipid homeostasis of PS or APP knock-out cells is rescued once they are incubated with Aβ peptides. Eventually the inverse cholesterol and sphingolipid regulation observed with the PS familial AD mutations could be resolved when the knock-out cells where studied in presence of Aβ42 or Aβ40. It was found that Aβ40 reduces the cellular 3-hydroxy-3-methyglutaryl coenzyme A reductase (HMGR) activity, the key regulated enzyme of cholesterol *de novo* synthesis. Strikingly, this enzyme is the target of the SREBP RIP mechanism extending the analogy between the original cholesterol regulation and that of the "AD" regulatory lipid cycle even further. Moreover the HMGR is also the direct target of statins, thus providing a molecular reasoning for the experimental statin therapy for AD.

Interestingly, Aβ42 has no effect on cholesterol *de novo* synthesis. But Aβ42 activates sphingomyelinases (SMases). SMases degrade sphingomyelin and therefore the increased Aβ42 production in PS familial AD mutations elevates, rather than lowers sphingomyelin levels. This also explains why cholesterol levels are still increased, although at a lower level, because the Aβ42 production apparently causes reduced Aβ40 levels and hence results in reduced HMGR inhibition [15]. Importantly, already the rather small physiological Aβ concentrations are sufficient to trigger this regulatory cascade and thus represent physiological events which occur in absence of AD too. This regulatory mechanism also extends beyond cholesterol and sphingolipids, because it contains feed-back mechanisms which are apparently involved in AD as well. The molecular layout of this feedback is known in far less detail, but the combined knowledge of gathered from molecular, clinical and epidemiological studies clearly highlights their importance for AD. *E.g.* cholesterol [2], cholesterol esters [29] and sphingomyelin [15] regulate Aβ production providing feed-back and similar evidence exists for some other lipids including gangliosides [30] and some n3-fatty acids, especially DHA and EPA [31, 32].

DHA

Fish oil or pure DHA decreased Aβ production in neuroblastoma and CHO cells in a dose dependent manner suggesting that DHA is involved in down regulation of the amyloidogenic pathway [32]. Furthermore dietary DHA reduces the production and accumulation of Aβ and decreases Aβ42 levels in aged Alzheimer mouse models [33]. In rats, administration of DHA had positive effects on the learning ability and suppressed the increase in lipid peroxide and reactive oxygen species levels in the cerebral cortex and hippocampus, suggesting an elevated anti-oxidative defence [34]. The intake of DHA or fish oil (contains DHA and EPA) rich diets by APP/PS1 transgenic mice resulted in decreased hippocampal Aβ levels [32]. The molecular mechanisms responsible for these effects remain largely unknown. Partly, this process might involve sub-cellular organization likely including lipid raft domains, which are affected by their relative content in cholesterol, saturated and non-saturated fatty acid containing lipids, governing structural integrity, membrane fluidity and functional properties in general. Unsaturated fatty acids increase fluidity, whereas cholesterol results in a stiffening of the respective membranes. Considering an average healthy EPA and DHA containing diet, high levels of DHA are incorporated into the human brain. DHA is the most abundant n-3 PUFA in the brain making up to 6% of the brain's dry weight [35] and is implicated in various functions. First of all, as an important membrane component, DHA is responsible for optimal membrane-protein interaction in signal transduction [36, 37]. Moreover, DHA enhances the gene expression in the brain including genes such as synuclein and serine palmitoyl transferase [38]. Long-term deficiency of DHA in the diet leads to cognitive impairment [39, 40]; however, the level of DHA in the brain and partially the cognitive performance can be restored by DHA administration. Additionally, DHA plays an important role in neurodegeneration. Lower level of DHA in the brain makes dendrites more vulnerable to h-amyloid [41] and impairs learning in h-amyloid-infused rats [42]. DHA is also the main antioxidant in the human brain. DHA is an essential fatty acid that can be acquired by several means. Either DHA is taken up from the diet or it is synthesized from a-linolenic acid and eicosapentaenoic acid (EPA), fatty acids that can only be acquired from diet [43, 44]. Direct uptake of dietary DHA and synthesis

from EPA are by orders of magnitude more effective than synthesis from linolenic acid through EPA [45]. Marine fish represent the most effective dietary source for DHA. Others DHA/EPA sources are meats like brain, liver and vessels. With the changes in dietary life-style it is clear from this list of sources that DHA uptake has drastically declined in the European population, especially fast within the last one or two decades. The n-6 to n-3 ratio was around 1-2 in the diet of our ancestors, and it is estimated to be now 10 or worse [46]. The brain has two major escape routes from this situation. DHA turnover is very slow, thus short term limited supply may not be problematic. If supply remains low or absent other fatty acids are used instead, including fatty acids like arachidonic acid (AA). This has several implications, including altered membrane properties and increased propensity to inflammation. Accordingly, once DHA supply increases, these substitute fatty acids are swiftly replaced by DHA.

Clinical perspective

DHA dependent Aβ production, neuronal function, cognitive performance and inflammation are all important factors for AD. It thus seems reasonable to assume that targeting lipids, diet or specifically DHA might provide protective or therapeutic potential for AD. Indeed this interpretation is supported by epidemiological and clinical data. A recent study indicated that Mediterranean diet might protect against AD [47] and other studies showed a negative correlation between fish consumption and AD [48-50].

Very recently one study was complete in which AD patients were given DHA. In this pilot trial patients at the initial clinical stage of AD (very mild AD) showed a stabilization of their cognitive performance while those given a placebo continued to decline over the study period of 12 month. Interestingly, those patients already at later stages of the disease showed no cognitive benefit [51]. Although statin treatment has not yet been tested with very mild AD patients these findings suggest that statins may provide a more effective treatment whereas DHA the more universally applicable alternative for prevention. However, the use of DHA for AD is a very recent development and further research may help to increase its effectiveness. In either case it is obvious from the limited number of patients thus far studied with either treatment that more large scale studies are needed. It is furthermore obvious from the preliminary data available that the future of AD therapy might reside with disease prevention or very early treatment to maximize effectiveness. This approach had been hampered severely by the inability to identify from the healthy elderly population those who are at highest risk to develop AD. Recent advances in molecular diagnostics have largely removed this issue [52].

The future challenges therefore will be to decipher the molecular pathways which link the neurodegeneration in Alzheimer's disease with lipids and based on that to optimize the therapeutic approach.

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