Nutrition and Alzheimer’s disease: pre-clinical concepts

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Alzheimer’s disease (AD) is a progressive condition characterized by neurodegeneration and the dense deposition of proteins in the brain. There is no cure for AD and current treatments usually only provide a temporary reduction of symptoms. There is thus a strong unmet need for effective preventative and therapeutic strategies and the potential role for nutrition in such strategies is rapidly gaining interest. An Alzheimer’s brain contains fewer synapses and reduced levels of synaptic proteins and membrane phosphatides. Brain membrane phosphatide synthesis requires at least three dietary precursors: polyunsaturated fatty acids, uridine monophosphate (UMP) and choline. Animal studies have shown that administration of these nutrients increases the level of phosphatides, specific pre- or post-synaptic proteins and the number of dendritic spines – a requirement for new synapse formation. These effects are markedly enhanced when animals receive all three compounds together. This multi-nutrient approach in animals has also been shown to decrease amyloid beta protein (Aβ) plaque burden, improve learning and memory through increased cholinergic neurotransmission and have a neuroprotective effect in several mouse models of AD. Whether these potential therapeutic effects of a multi-nutrient approach observed in animal models can also be replicated in a clinical setting warrants further investigation.

Introduction

Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by progressive impairment of episodic memory leading to global dementia. Various pathogenic mechanisms have been proposed to underlie AD, including the accumulation of amyloid beta protein (Aβ) forming neuritic plaques, and the somatodendritic build-up of hyperphosphorylated tau protein forming neurofibrillary tangles [1]. The brains of people with AD are also deficient in cholinergic neurons and, generally, in synapses. Synapse loss tends to be greatest in the medial temporal lobe and the hippocampus, a locus of memory formation.

Knowledge of the neurotransmitter deficits in AD has led to the development of drugs which provide a temporary amelioration of symptoms in some patients. Research is ongoing to develop drugs which may suppress amyloid plaque development [2]. Thus there remains a strong unmet need for effective therapeutic strategies, which may or may not be based on modifying neurotransmitter metabolism, the formation of Aβ or hyperphosphorylated tau.

Diet and nutrition have increasingly been recognized as potentially-important factors influencing the susceptibility to and natural history of AD [3,4] and a potential role for nutrition in the management of AD seems likely [5,6]. This mini-review describes the pre-clinical evidence that specific nutrients may have therapeutic utility in AD – particularly affecting the synthesis and integrity of brain membranes. It focuses on the roles of supplemental docosahexaenoic acid (DHA), uridine-5'-monophosphate (UMP) and choline in promoting the synthesis of neuronal membranes and thereby enhancing synaptic transmission in the mammalian brain [7,8].

Lipids and synaptic function

Accelerated loss of neuronal membranes is a common finding in people with AD [9]. Post-mortem ultrastructural investigations of the brains of people with AD have consistently demonstrated a decline in synaptic density in the neocortex and dentate gyrus which does not appear to be related to the ageing process. Recent transmission electron microscopic analysis of the total number of synapses in the outer molecular layer of the human dentate gyrus revealed a strong...
reduced risk of AD in the general population [126-281].

DHA is significantly lower in the plasma [23,24] and established that omega-3 fatty acid levels (including DHA) are extensively modulated by dietary lipids [21] and DHA is essential role of DHA in the normal maintenance of brain functions, including ion-channels, receptors and enzymes, which are essential for neurotransmission. The membrane lipids and their peroxidation products are important participants in central nervous system function via such roles as signal transduction molecules and neuronal structural components.

Essential fatty acids, which must be obtained from the diet, are critically important for the structural integrity of neuronal membranes [12]. The most abundant fatty acid in the brain is DHA, an omega-3 polyunsaturated fatty acid (PUFA) that is present in approximately 30-40% of the phospholipids of the cerebral cortex gray matter and photoreceptor cells of the retina [13]. DHA is particularly concentrated in synaptic membranes [14] and in myelin sheaths [15]. Human and animal studies have demonstrated the essential role of DHA in the normal maintenance of brain functions, including synaptic plasticity, neurotransmission and vision [16,17]. For example, DHA is responsible for optimal membrane-protein interaction in signal transduction [18], enhancing expression of genes such as synuclein [19] and is essential for brain development and synaptogenesis [20].

The brain is generally well-protected against external blood-borne influences by the blood–brain barrier (BBB). However, cerebral fatty acid composition is extensively modulated by dietary lipids [21] and DHA is taken up by the brain in preference to other fatty acids [22]. This has significant implications as it is well established that omega-3 fatty acid levels (including DHA) are significantly lower in the plasma [23,24] and brain [25] of patients with AD, compared with controls, and increasing dietary intake of DHA is associated with reduced risk of AD in the general population [26-28].

Phosphatidylcholine (PC) is the most abundant phospholipid in neuronal membranes [29]. Its synthesis in brain utilizes, besides glucose, three dietary compounds obtained from the circulation: choline; a pyrimidine such as uridine; and a PUFA such as DHA [30]. Each of these compounds crosses the BBB [31-34] and synthesis of PC is restricted by the availability of substrates and rate-limiting enzymes involved in the biosynthesis of phosphatidylcholine (PC). Uridine readily enters the brain yielding UTP that can be converted to CTP. CTP then reacts with phosphocholine to form CDP-choline, which combines with DAG [preferentially species containing omega-3 polyunsaturated fatty acid (PUFA) like docosahexaenoic acid (DHA)] to form PC. In rats, cytidine is the major circulating pyrimidine; in humans and gerbils the primary circulating pyrimidine is uridine. CK, choline kinase; CT, CTP: phosphocholine cytidylyl transferase; CPT, CDP-choline: 1, 2-diacylglycerol choline phosphotransferase.

Figure 1 Biosynthesis of phosphatidylcholine (PC). Uridine readily enters the brain yielding UTP that can be converted to CTP. CTP then reacts with phosphocholine to form CDP-choline, which combines with DAG [preferentially species containing omega-3 polyunsaturated fatty acid (PUFA) like docosahexaenoic acid (DHA)] to form PC. In rats, cytidine is the major circulating pyrimidine; in humans and gerbils the primary circulating pyrimidine is uridine. CK, choline kinase; CT, CTP: phosphocholine cytidylyl transferase; CPT, CDP-choline: 1, 2-diacylglycerol choline phosphotransferase.

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of B-vitamins might stimulate neuronal PC formation. Based on this capacity for PC biosynthesis, several animal studies have investigated the effect of supplementing the diet with nutrients to promote neuronal membrane biosynthesis.

**Nutrients increase membrane synthesis and synapse formation**

Experimental studies in gerbils have demonstrated that dietary supplementation with uridine (as its monophosphate, UMP) increases plasma and brain uridine levels with a subsequent increase in brain levels of CDP-choline, the immediate precursor to PC [42]. Further *in vitro* and *in vivo* studies have demonstrated that formation of neuronal membranes is promoted by increasing the availability of UMP and choline, resulting in an increase in the number and size of axonal neurites [44,45]. This effect is mediated in part by a direct action of uridine and its phosphorylated metabolites (e.g. UTP) on brain P2Y2 receptors, and is blocked by drugs that antagonize those receptors [43].

Importantly, when animals receive DHA as well as uridine and choline, the neuromodulatory actions are markedly enhanced [7,41,44]. For example, gerbils fed a choline-containing diet in which UMP, DHA or a combination were supplemented for four weeks, exhibited a 13–22% rise in PC with uridine and choline alone, and by 45% with the combination of uridine, choline and DHA, suggesting a synergistic effect of the nutrients [7].

The relevance of these increases in phospholipid synthesis has been demonstrated in *in vitro* cell culture experiments [43]. Studies in nerve growth factor (NGF) differentiated PC-12 cells have shown that incubation with uridine significantly increases the number of neurites per cell in a dose-dependent manner. This was accompanied by increased neurite branching and elevated levels of the neurite protein neurofilament M and neurofilament 70. Importantly, uridine treatment also increased intracellular levels of CTP, suggesting that the effects on neurite outgrowth were mediated by enhanced PC synthesis. Effects of uridine on neurite outgrowth were replicated in cocultures of hippocampal embryonic neurons (E18) and astrocytes (M. Verheijen, pers. com. [46]). In addition, others have found that incubating PC12 cells with DHA promoted, whilst the n-6 PUFA arachidonic acid suppressed, NGF-induced neurite outgrowth [47]. Fujii *et al.* have also shown that enriching the medium (using cells obtained from the dorsal root ganglia of mice) with vitamin B complex (vitamin B1, B6 and B12) increases neurite outgrowth [48].

These observations have been confirmed *in vivo* by Sakamoto *et al.* [8] using gerbils whose standard choline-containing diets were supplemented with DHA or a combination of DHA and UMP. Oral supplementation with DHA (or eicosapentaenoic acid, another omega-3 fatty acid, but not by the omega-6 fatty acid arachidonic acid) was shown to substantially increase the number of dendritic spines in the hippocampus, and this effect was almost doubled when animals were co-supplemented with UMP (Fig. 2). The increase in dendritic spines was accompanied by parallel increases in membrane phosphatides and levels of the pre- and post-synaptic proteins syntaxin-3, PSD-95 and synapsin-1 (but not in those of a ubiquitous structural protein beta-tubulin) indicating an increase in quantity of synaptic membrane [41]. The supplementation of all three nutrients in the diet has also been shown to improve hippocampus-dependent cognitive behaviours in rats reared in a socially deprived environment [45].

Dendritic spines mediate most excitatory connections in the brain and are thought to directly reflect the number of excitatory synapses in the central nervous system. Hence, oral DHA may promote neuronal membrane synthesis to increase the number of synapses, particularly when co-administered with UMP to a choline-containing diet.

**Nutrients Improve learning and memory**

Membrane PC is also an important source of choline in the biosynthesis of acetylcholine, a major neurotransmitter.
involved in learning and memory [49]. Microdialysis studies in freely moving rats have shown that administration of UMP increases acetylcholine levels in the striatum and striatal extracellular fluid in both aged and young rats [50]. Importantly, no effects on acetylcholinesterase activity were observed, indicating that the effect was a result of increased synthesis of acetylcholine. These findings are important as the pathophysiology of AD involves the preferential loss of cholinergic neurons in the basal forebrain. The behavioural relevance of these data is underlined by studies of rats reared under impoverished environmental conditions [51]. In these studies, exposure of rats to an impoverished environment leads to impaired hippocampal-dependent learning and memory which could be prevented upon supplementation with UMP. Similarly, concomitant administration of uridine and choline improved selective attention and spatial learning (as assessed by the five-choice serial reaction time task and the Morris water maze, respectively) in spontaneously hypertensive rats (SHR), which exhibit a well-defined cognitive deficit (Fig. 3A) [52]. Administration of DHA has also been reported to improve Morris water maze learning in APP/PS1 transgenic mice (Fig. 3B) [53]. In this model, reducing dietary intake of omega-3 fatty acids was shown to result in an 80–90% loss of the p85alpha subunit of phosphatidylinositol 3-kinase and in the post-synaptic actin-regulating protein drebrin. Treatment of omega-3 PFA-restricted mice with DHA protected against these effects, thereby improving cognitive function.

**Nutrients and amyloid precursor protein processing**

According to the amyloid hypothesis of AD, accumulation of Aβ in the brain is the primary influence driving AD pathogenesis [54]. Because of the role of dietary omega-3 fatty acids in membrane fluidity, it has been suggested that they may alter enzymatic activity of the secretase enzymes involved in the processing of the amyloid precursor protein [55]. In vitro studies using ovarian hamster cells transfected with human APP have shown that, on incubation with increasing dosages of DHA, changes in the omega-3/omega-6 ratio of neuronal membranes (as DHA is incorporated into the membrane) correlated with a decrease in cellular amyloid beta (Aβ) production [56]. The potential of DHA to prevent both production and accumulation of Aβ-peptide has been demonstrated in a number of animal studies. Dietary supplementation of DHA in APP transgenic mice significantly reduced amyloid plaque burden by 40% in the hippocampus and parietal cortex in aged transgenic animals (Fig. 4) [57] as well as decreasing the number of activated microglia in the hippocampus [58].

Inclusion of DHA into the diet of rodent models of AD has also been shown to increase exploratory activity in APP/PS1 transgenic mice [58] and improve spatial cognition learning ability in Aβ-infused rats [32, 59]. Most recently, a study in a double-transgenic APP/PS1 mouse model of AD found that, compared with a normal diet, mice receiving a diet enriched with DHA, UMP, choline and minerals showed a reduction in Aβ plaques which was accompanied by an attenuation of

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neuronal damage [60]. Importantly, mice receiving these dietary enhancements as individual supplements did not demonstrate significant changes in Aβ plaques or brain cell damage, suggesting that a multi-nutrient diet is more effective than a single-nutrient enriched diet.

Further studies in a mouse model of AD have demonstrated the neuroprotective benefits of a multi-nutrient enriched diet. Infusion of Aβ into the lateral ventricles of mice has been shown to reduce choline acetyltranserase levels by 30–40% and reduce locomotor activity in the open field. This decrease in choline acetyltransferase activity can be completely restored when mice receive the multi-nutrient enriched diet (including: DHA, EPA, UMP, choline, phospholipids, antioxidant and B vitamins) and behavioural deficits are normalized [61].

Conclusions

It is clear that dietary supplementation with specific nutrients has beneficial effects in animal models of AD. A striking feature of the role of the nutrients discussed in this review is the apparent synergistic effects of multiple nutrients on AD pathophysiology and cognitive function. Specifically, the neuromodulatory actions of DHA, uridine and choline in promoting new neuronal membrane and synapse formation are markedly enhanced when animals receive all three compounds together [7,8,41,62]. As nutrients may have additive effects and may interact to produce their beneficial effect, combining multiple nutrients may be more effective than single nutrient supplementation.

This may also translate into clinical strategies. For example, one of the first studies to evaluate the effect of multiple nutrients in an AD population found that adherence to a Mediterranean diet (characterized by high intake of vegetables, legumes, fruits, cereals and unsaturated fatty acids and fish, and low intake of saturated fatty acids) not only reduced the risk of developing AD, but also favourably affected the course of the disease [3,4]. Superior adherence to a Mediterranean diet was associated with lower mortality in AD and led the authors to suggest a possible dose–response effect.

There is a clear unmet medical need for novel approaches to the management of AD. Whilst there are...
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limitations in applying the findings of pre-clinical models to the clinic, the growing evidence from pre-clinical studies indicates that a combination of nutrients is more effective than single nutrients, and thus a multi-nutrient approach appears to be a promising clinical approach in the management of AD. A multi-nutrient intervention would represent a completely novel approach to AD management. Whether the potential therapeutic effects of combined nutrients observed in animal models can also be replicated in a clinical setting warrants further investigation.

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Conflicts of interest

PJ Kamphuis is an employee of Danone Research; RJ Wurtman is a professor at MIT, which owns patents and has filed patent applications relating to the uses of compounds described in this review to increase synapse formation and to treat diseases associated with the loss of synapses. RW is also a paid consultant to Danone on scientific matters.

References