A multi nutrient concept to enhance synapse formation and function: science behind a medical food for Alzheimer's disease

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Abstract: *Alzheimer's Disease (AD) is the leading cause of dementia. Epidemiological studies suggest that AD is linked with poor status of nutrients including DHA, B-vitamins and the vitamins E and C. Ongoing neurodegeneration, particularly synaptic loss, leads to the classical clinical features of AD namely, memory impairment, language deterioration, and executive and visuospatial dysfunction. The main constituents of neural and synaptic membranes are phospholipids. Supplemenation of animals with three dietary precursors of phospholipids namely, DHA, uridine monophosphate and choline, results in increased levels of brain phospholipids, synaptic proteins, neurite outgrowth, dendritic spines formation (i.e. the anatomical precursors of new synapses) and an improvement in learning and memory. Other nutrients act as co-factors in the synthesis pathway of neuronal membranes. For example B-vitamins are involved in methylation processes, thereby enhancing the availability of choline as a synaptic membrane precursor. A multi-nutrient concept that includes these nutrients may improve membrane integrity, thereby influencing membrane-dependent processes such as receptor function and amyloid precursor protein (APP) processing, as shown by reduced amyloid production and amyloid β plaque burden, as well as toxicity. Together, these insights provided the basis for the development of a medical food for patients with AD, Souvenaid®, containing a specific combination of nutrients (Fortasyn™ Connect) and designed to enhance synapse formation in AD. The effect of Souvenaid on memory and cognitive performance was recently assessed in a proof-of-concept study, SOUVENIR I, with 212 drug-naïve mild AD patients (MMSE 20-26). This proof-of-concept study demonstrated that oral nutritional supplementation with Souvenaid® for 12 weeks improves memory in patients with mild AD. To confirm and extend these findings, we have designed and initiated three additional studies. Two of these studies will be completed in 2011; Souvenir II, a 24-week European study, with 259 drug-naïve mild AD patients (MMSE≥20) and S-Connect, another 24-week study, with 527 mild-to-moderate AD patients (MMSE 14-24) using AD medication conducted in the US. The third is the EU-funded LipiDiDiet study, a 24-month study, which will enrol 300 people with prodromal AD to assess the effect on memory performance.*

Key words: *Alzheimer's disease, nutrition, souvenaid, synapse, membrane, phospholipids*

Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder and is the leading cause of dementia. In 2010, there were an estimated 35.6 million AD sufferers worldwide and, with the global ageing population, the estimate is expected to increase to 115.4 million by 2050 (ADI, 2010). Age is the primary risk factor (von Strauss *et al.*, 1999; Citron, 2002), while the primary genetic risk factors include family history of AD (for familial AD) and presence of the apolipoprotein E-ε4 genotype (apoE4, for sporadic AD) (Fratiglioni *et al.*, 1993; Seshadri *et al.*, 1995). Furthermore, increasing epidemiological evidence suggests diet as one of the most important modifiable risk factors (Engelhart *et al.*, 2002; Morris *et al.*, 2003). Other aspects of disease aetiology, including vascular and psychosocial

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factors, are under investigation (Qiu *et al.*, 2009).

The exact cause of AD remains unknown, despite decades of research guided by several hypotheses based upon differing brain pathologies. Amyloid plaques, neurofibrillary tangles and progressive loss of neurons are characteristic hallmarks of AD that allow for post-mortem histological confirmation of the condition. However, severe synaptic loss and/or reduced synaptic activity and connectivity in specific brain areas occurs early in the disease process resulting in the classic clinical features of AD: memory impairment, language deterioration, and executive and visuospatial dysfunction (Terry and Katzman, 2001; Scheff and Price, 2006). Soluble beta-amyloid (Aβ) protein oligomers have been proposed as the pathogenic agents that induce loss of synaptic and dendritic spines in AD (Haass and Selkoe, 2007; Shankar *et al.*, 2007; Shankar *et al.*, 2008; Freir *et al.*, 2010). It has been reported that symptomatic dementia occurs when there is an approximately 40% reduction in cortical synapses compared with age-matched healthy adults (Terry *et al.*, 1991). Synapse loss is the strongest structural correlate with cognitive performance in AD, even more so than the prevalence of plaques or tangles (Terry *et al.*, 1991; Terry, 2006). Therefore, improving synaptic formation and function may well be a target for intervention in AD.

Enhancing neuronal membrane and synapse formation and function with nutrients

Synapses are highly specialized membrane structures that form the contact points enabling information exchange between neurons. Synapses typically consist of an axonal presynaptic nerve terminal, a synaptic cleft, and a postsynaptic membrane, usually on a dendrite or cell body. New brain synapses form when a postsynaptic structure, the dendritic spine, interacts with a presynaptic nerve terminal (Toni *et al.*, 2007). Since dendritic spine growth precedes synapse formation and new synapses form preferentially onto existing boutons (Knott *et al.*, 2006), the rate of synaptogenesis depends, at least in part, on the numbers of available dendritic spines. The membranes of dendritic spines and synapses are composed of phospholipids and contain synaptic proteins. Phospholipids are generated primarily via the cytidine diphosphate (CDP)-choline and CDP-ethanolamine pathways of the Kennedy cycle (Kennedy and Weiss, 1956). The formation of phospholipids in the brain is dependent upon an adequate supply of circulating dietary compounds, because nutrients increasing the substrate-saturation of low-affinity enzymes that synthesize the phospholipids (Wurtman *et al.*, 2009). Thus, increasing brain phospholipid levels by dietary means could increase the quantity of the brain membranes. Indeed, animals given three of these precursors, uridine, DHA, and choline, develop increased levels of brain phospholipids (Wurtman *et al.*, 2006; Cansev and Wurtman, 2007; Sakamoto *et al.*, 2007a; Cansev *et al.*, 2008; Holguin *et al.*, 2008b; Holguin *et al.*, 2008a; Cansev *et al.*, 2009). For example, gerbils receiving a daily diet containing choline (0.1%) and uridine monophosphate (UMP, 0.5%) or DHA (300 mg/kg) by gavage for 4 weeks showed up to a 22% increase in brain phosphotidylcholine (PC) levels. Interestingly, combining the three nutrients (choline, UMP, DHA) increased brain PC levels by 45%, and the other phospholipid classes by up to 74% (Wurtman *et al.*, 2006). Thus, while providing the single nutrients clearly increases brain phospholipid levels, the largest increases are obtained when all three precursors are combined. Combined supplementation of choline, UMP, and DHA was also found to increase brain levels of specific proteins such as synapsin-1, PSD-95, and syntaxin-3, known to be localized within presynaptic and postsynaptic membranes. However, dietary intervention had no effect on the cytoskeletal protein beta-tubulin, indicating a selective increase in synaptic membrane. The effects of DHA and UMP supplementation on dendritic spine number were examined in adult gerbils treated daily for 1-4 weeks; animals received single or combined compounds (Sakamoto *et al.*, 2007b). DHA alone caused dose-related increases in spine density, accompanied by parallel increases in membrane phospholipids and in specific pre- and postsynaptic proteins; its effect was doubled if animals also received UMP. Administration of DHA, UMP, and choline to normal adult animals improved hippocampus-dependent cognitive behaviors in rats (Teather and Wurtman, 2006; Holguin *et al.*, 2008b) and gerbils (Holguin *et al.*, 2008a). These findings suggest that a nutrient supplementation that increases synaptic membrane and dendritic spine formation in the brain enhances cognitive processes associated with synaptic functioning.

B-vitamins, phospholipids and antioxidants serve as co-factors by enhancing the availability of these precursors (van Wijk *et al.*, 2011). Combined dietary enrichment with these nutrients has also been shown to influence membrane-dependent processes, e.g. reducing amyloid precursor processing (APP) pathways and receptor function. This, in turn, could result in reduced Aβ production, plaque burden and Aβ toxicity (Broersen *et al.*, 2007). Additional data suggest that this multi-nutrient combination protects the cholinergic system against $A\beta_{42}$-induced toxicity (de Wilde *et al.*, 2011b). In support of this, epidemiological and cohort studies indicate that a diet rich in omega-3 fatty acids, B-vitamins, and antioxidants decreases the risk of AD (Barberger-Gateau *et al.*, 2007; Luchsinger *et al.*, 2007), while others have reported lower plasma levels of these nutrients in patients with AD compared with cognitively intact age-matched controls (Shatenstein *et al.*, 2007).

Proof of concept and future directions

The observations described in the previous section led to the development of Souvenaid®, a multi-nutrient drink designed to deliver the supporting nutrients to improve synaptic membrane formation and function in patients with AD. This medical food contains a specific formulation of nutrients registered as Fortasyn™ Connect (including DHA, EPA, phospholipids, choline, UMP, vitamin B12, B6, and folate, vitamins C and E and selenium) (Scheltens *et al.*, 2010). The efficacy and tolerability of Souvenaid was recently assessed in 225 drug-naïve mild AD patients (MMSE 20-26) in a multicenter, controlled, proof-of-concept Souvenir I study (Scheltens *et al.*, 2010). The study was a 12-week randomized, double-blind, controlled, parallel-group, multi-centre, multi-country study, with a 12-week similarly designed exploratory extension period, in which patients received the same study product as in the first 12 weeks

of the study. Patients were randomly assigned to active product (Souvenaid) or an isocaloric control drink, taken once-daily for 12 weeks. After 12 weeks, patients were invited to continue in an optional 12-week extension study. Blood samples from patients completing the 24 week program were used to analyze plasma or erythrocyte levels of nutrients. Compliance was excellent (94%) and this was biochemically confirmed by marked increase of DHA and EPA levels in the erythrocyte membranes and reduced plasma homocysteine. The results of the study also demonstrated a very favorable safety profile for Souvenaid with no differences between the Souvenaid and control group in the incidence of either adverse events or serious adverse events. Furthermore, no difference was observed between the active and control group in biochemical safety markers of liver and renal function). At 12 weeks, a significant improvement in the Wechsler Memory Scale-revised (WMS-r) delayed verbal recall score (co primary outcome measure) was noted in the Souvenaid group compared with control ($p = 0.021$). The other co-primary outcome measure, Modified Alzheimer's Disease Assessment Scale–cognitive (ADAS-cog) subscale, and secondary outcome scores (e.g., Clinician Interview Based Impression of Change plus Caregiver Input, 12-item Neuropsychiatric Inventory, Alzheimer's disease Co-operative Study–Activities of Daily Living, Quality of Life in Alzheimer's Disease) were unchanged. In a pre-specified subgroup analysis of patients with very mild AD (baseline MMSE 24-26), daily intake of Souvenaid for 12 weeks resulted in an improved memory performance compared with the control. Both immediate and delayed verbal memory scores were significantly improved ($p = 0.011$) in this pre-specified sub-group. In addition to effects on delayed and immediate memory performance at 12 weeks, a significant improvement in the immediate but not in the delayed verbal recall task was noted in the active group compared with control after 24 weeks intervention. The Souvenir I study was primarily designed to study effects after 12 weeks intervention (de Wilde *et al.*, 2011a). It should be taken into account that the extension period had an exploratory character and aimed to obtain additional efficacy, tolerability and safety data during a longer period. Post hoc analysis indicated a significant positive effect on Activities of Daily Living (ADL) after 12 weeks for the subgroup of AD patients with a body mass index (BMI) below the mean of the total group (Kamphuis *et al.*, 2011b). Finally, in secondary analysis a significant treatment effect on ADAS-cog was shown in patients with ''high'' baseline ADAS-cog, but not in patients with 'low' baseline ADAS-cog (Kamphuis *et al.*, 2011a).

To confirm and extend the initial findings of the efficacy and safety of Souvenaid, three additional randomized double-blind controlled studies were started in 2009. The 'S-Connect' study (NTR1683) is a 24-week randomized, controlled, double-blind, study in $>$ 500 mild-to-moderate AD subjects (MMSE 14-24) using AD medication across 48 sites in the United States. The 'Souvenir II' study (NTR1975) is a 24-week randomized, controlled, double-blind, European study in 226 drug naïve mild AD subjects (MMSE $\geq$ 20) across 27 centers in The Netherlands, Belgium, Germany, France, Spain and Italy. Finally, the ''LipiDiDiet study'' (NTR1705), is a 24-month randomized, controlled, double-blind, study in 300 prodromal AD subjects (MMSE $\geq$ 24). Results of these studies are expected to be available between 2011 and 2014.

In conclusion, Souvenaid is a multi-nutrient drink designed to improve the formation and function of synapses in AD. A proof-of-concept study indicated that memory performance in drug naïve mild AD was improved. Clinical studies to confirm and extend this finding are ongoing.

Disclosure statements

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