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Neuroprotective and cognitive enhancing effects of a multi-targeted food intervention in an animal model of neurodegeneration and depression

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ABSTRACT

Rising neurodegenerative and depressive disease prevalence combined with the lack of effective pharmaceutical treatments and dangerous side effects, has created an urgent need for the development of effective therapies. Considering that these disorders are multifactorial in origin, treatments designed to interfere at different mechanistic levels may be more effective than the traditional single-targeted pharmacological concepts. To that end, an experimental diet composed of zinc, melatonin, curcumin, piperine, eicosapentaenoic acid (EPA, 20:5, n-3), docosahexaenoic acid (DHA, 22:6, n-3), uridine, and choline was formulated. This diet was tested on the olfactory bulbectomized rat (OBX), an established animal model of depression and cognitive decline. The ingredients of the diet have been individually shown to attenuate glutamate excitoxicity, exert potent anti-oxidant/anti-inflammatory properties, and improve synaptogenesis; processes that all have been implicated in neurodegenerative diseases and in the cognitive deficits following OBX in rodents. Dietary treatment started 2 weeks before OBX surgery, continuing for 6 weeks in total. The diet attenuated OBX-induced cognitive and behavioral deficits, except long-term spatial memory. Ameliorating effects of the diet extended to the control animals. Furthermore, the experimental diet reduced hippocampal atrophy and decreased the peripheral immune activation in the OBX rats. The ameliorating effects of the diet on the OBX-induced changes were comparable to those of the NMDA receptor antagonist, memantine, a drug used for the management of Alzheimer's disease. This proof-of-concept study suggests that a diet, which simultaneously targets multiple disease etiologies, can prevent/impede the development of a neurodegenerative and depressive disorders and the concomitant cognitive deficits.

1. Introduction

Rising neurodegenerative and depressive disease prevalence combined with a lack of effective pharmaceutical treatments has created an urgent need for novel therapeutic approaches. Neurodegenerative disorders are multifactorial in origin with a complex set of pathological pathways (Dauer and Przedborski, 2003; Anand et al., 2012; Chopra et al., 2011). Therefore, simultaneous manipulation of these pathways may exert higher or better therapeutic efficacy than a single approach alone (Wollen, 2010). To prevent a disease onset, most people will probably prefer the daily intake of natural food supplements above the daily intake of drugs (Rozin et al., 2004). Hence, dietary components have emerged as potential preventatives and/or treatments for neurodegenerative and depressive disorders. Alzheimer's disease, in particular, is being targeted with dietary treatments due to the limited adverse side effects as compared to pharmacological interventions (Wollen, 2010; Kamphuis and Wurtman, 2009; Scheltens et al., 2013). Clinical studies show higher efficacy of the dietary interventions especially at the early phases of the disease, whereas at later stages

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treatments are rather disappointing (Kamphuis and Scheltens, 2010)

The aim of this study was to investigate the neuroprotective effects of a dietary intervention in olfactory bulbectomized (OBX) rats, an animal model of neurodegeneration and depression. Removal of the olfactory bulbs leads to neuronal degeneration in several different brain areas and cognitive decline (Wrynn et al., 2000; Song and Leonard, 2005; Borre et al., 2012a). Although the initial mechanism of neurodegeneration induction in the OBX rats differs from that in neurodegenerative diseases in humans, the secondary changes, such as NMDA-receptor-mediated excitotoxicity, impaired structural plasticity, and neuroinflammation, have much in common with human neurodegenerative diseases.

In this proof-of-concept study it was hypothesized that a combination diet of unique nutritional ingredients, individually known to be (partly) beneficial in the OBX model, would effectively impede cognitive decline and neurodegeneration when chronically administered starting two weeks before the surgery. The diet was composed of zinc, melatonin, curcumin, piperine, eicosapentaenoic acid (EPA 20:5, n-3), docosahexaenoic acid (DHA, 22:6, n-3), uridine, and choline (Table 1). These ingredients have been individually shown to attenuate glutamate excitoxicity, exert potent antioxidant/anti-inflammatory properties, or improve synaptogenesis; processes that all have been implicated in neurodegenerative diseases and in the cognitive deficits following OBX in rodents. Some of these ingredients have already been tested in OBX rats (Nowak et al., 2003; Tasset et al., 2010; Song et al., 2009), but in the studies so far, dietary interventions started several weeks after surgery-a time point at which the OBX-induced secondary neurodegenerative processes have been completed (Song and Leonard, 2005). Clinical data combined with our previous studies (Borre et al., 2012a,b,c) suggest a higher efficacy in early intervention rather than late treatment. Therefore, current study employed an alternative approach of commencing treatment regiment prior to the OBX and exploring prophylactic/preventive therapies.

Zinc is a potent antagonist of the NMDA receptor (Dingledine et al., 1999; Smart et al., 1994) and has been shown to modulate glutamate neurotransmission (Morris and Levenson, 2012; Watt et al., 2010) and to be neuroprotective (Bancila et al., 2004). Under normal conditions, zinc plays a critical role in learning and memory (Mocchegiani et al., 2005; Bitanihirwe and Cunningham, 2009). Curcumin has antioxidant and anti-inflammatory properties (Cole et al., 2007; Braidy et al., 2010; Ishrat et al., 2009). Since curcumin has a poor absorption rate, which undermines its bioavailability, piperine has been added. Piperine is a major alkaloidal constituent of black pepper. It is a powerful inhibitor of hepatic and intestinal glucuronidation, and increases the bioavailability of many drugs including curcumin (Atal et al., 1985; Shoba et al., 1997). In addition, piperine possesses potential antidepressant effects by inhibiting monoamine oxidase A and B, improves cognitive performance, and has anti-inflammatory and anti-oxidative properties (Chonpathompikunlert et al., 2010; Wattanathorn et al., 2008; Selvendiran et al., 2003). Melatonin is a hormone involved in sleep regulation and a free radical scavenger. It is able to pass the blood–brain barrier, making this compound a potential neuroprotective agent (Reiter et al., 2000; Olcese et al., 2009; Ramirez-Rodriguez et al., 2011). UMP, choline and the n-3 fatty acids EPA and DHA were added as well. These compounds stimulate neurite outgrowth, dendritic spine formation and are necessary for brain phosphatide synthesis (Wurtman, 2011). Recently, it was shown that feeding mice for 3 month with DHA alone is already sufficient to increase n-3 fatty acids in brain (Broersen et al., 2013). Importantly, a diet containing, in addition to DHA, UMP, choline, some vitamins and selenium reduced the amyloid plaque burden in Alzheimer's disease transgenic, indicating that increased amounts of n-3 fatty acids in the brain lipids by itself are not sufficient? It is the combination of relevant nutrients that has the relevant biological effect. On the other hand, animal studies have shown that n-3 poly-unsaturated fatty acid supplementation alone is already sufficient to suppress inflammation (James et al., 2000; Calder, 2001), attenuate the stress response and improve cognition (Song et al., 2009, 2009; Ikemoto et al., 2001). Importantly, it has been shown in recent clinical trials that Souvenaid®, a medicinal food mixture that contains n-3 fatty acids, choline, and UMP as main ingredients, improves memory performance in drug-naïve patients with mild Alzheimer's disease (Kamphuis et al., 2011; Scheltens et al., 2013). Concentrations of various supplements included in the experimental diet were derived from the literature where these concentrations were effective in attenuating cognitive and behavioral deficits in either OBX rodents, other animal models of neurodegeneration or clinical setting (Souvenaid®). For example, zinc showed antidepressant and cognition enhancing effects in the OBX (Nowak et al., 2003) Melatonin normalized OBX-induced aberrant changes in the open field (Tasset et al., 2010). Curcumin administration reduced OBX-induced hyperactivity and fear memory deficits (Xu et al., 2005). EPA treatment attenuated OBX-induced behavioral and cognitive deficits (Song et al., 2009). Piperine significantly improved spatial memory and neurodegeneration in AD animal model (Chonpathompikunlert et al., 2010). Souvenaid® improves memory performance in patients with mild Alzheimer's disease (Kamphuis et al., 2011; Scheltens et al., 2013).

The above-described diet was given to the rats starting 2 weeks before surgery and continued for 6 weeks. Animals were assessed in behavioral and cognitive paradigms at several time points during the treatment regimen. To compare the efficacy of the experimental diet to the existing pharmacological treatment for Alzheimer's disease, a separate group of animals was treated with memantine, an NMDA receptor antagonist. To ensure OBX-induced behavioral and cognitive deficits were independent of anosmia, a separate group of animals were made anosmic by destroying the nose epithelium with a $ZnSO_4$ infusion. After the completion of the behavioral screening, the modulating effects of experimental diet on molecular, immune and cellular parameters in the brain and spleen were evaluated. Rats were tested at several time points during the treatment regimen in the open field (assessing locomotor activity of the animals), passive avoidance (a task assessing fear memory), T-maze (a task assessing short-term spatial memory) and hole board (a task assessing spatial and reference memory) paradigms. Because hippocampus is involved in various cognitive functions and is affected in neurodegenerative disorders and OBX animals, we analyzed hippocampi for atrophy and cell loss using nissl staining in order to assess OBX-induced cell in hippocampus. To assess if experimental diet attenuated OBX-induced abnormal inflammatory response, a key feature in various neurodegenerative disorders, we performed histological examination of the spleen.

Table 1
Experimental and control diet compositions. Rats were given 20 g of the diet per day. The delivered dose is on a mg/kg of food.

Active ingredient	Control (AIN-93) g/kg diet	Experimental diet
Zinc	0.03	1.63
Curcumin	0	0.25
Piperine	0	0.06
Melatonin	0	0.03
Choline	1.09	9.5
Uridine	0	15.48
Soy oil	7%	3% soya+4% tuna oil (25% DHA/6% EPA)