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NEUROPROTECTIVE EFFECTS OF LOW FAT-PROTEIN DIET IN THE P301L MOUSE MODEL OF TAUOPATHY

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Abstract—Tauopathies are a class of neurodegenerative diseases associated with the pathological aggregation of tau protein in the human brain. Although numerous studies in mouse models of Alzheimer disease (AD) have shown a correlation among diet, beta-amyloid and AD onset, little is known about the impact of diet on Tau. We investigated whether a low fat-protein diet (LFPD) may improve lifespan, cognitive and locomotor activity in P301L-tg mouse model of tauopathy. Our data indicate that LFPD has a beneficial effect on these parameters. Tg mice fed with standard diet shown a decrease in body weight, food intake and survival rate if compared to wild type animals. In contrast, LFPD counteracted weight loss, increased mortality and ameliorated cognitive and locomotor performances in tg mice. LFPD also reduced the abnormal accumulation of agglomerates of P-Tau (pathological features of tauopathies) and the expression of apoptotic markers (i.e., TUNEL immunopositive neurons) in the prefrontal cerebral cortex and hippocampus of P301L-tg mice. Interestingly, some of these effects are sex-dependent. For instance, tg females, but not males, fed with LFPD had a significant increase of body weight and a reduction of P-Tau agglomerates compared to tg fed with standard diet. These changes correlated with a more pronounced improvement of cognition and locomotor activity in females than in male tg fed with LFPD. Altogether, these results suggest a sex dependent neuroprotective effect of LFPD in P301L-tg mice, suggesting that lifestyle intervention strategies may be clinically relevant for delaying the onset of cognitive impairment and dementia, especially in females.

Key words: diet, tauopathy, sex difference, hyperphosphorylated Tau, neuronal death, neuroprotection.

INTRODUCTION

Tauopathies are neurodegenerative diseases including several forms of dementia, such as frontotemporal dementia, progressive supranuclear palsy and Alzheimer disease (AD) (Nasreddine et al., 1999; Iqbal et al., 2005). Age, sex and nutrition are the major risk factors for dementia (Launer et al., 1999). In particular, recent epidemiological studies show specific association between nutritional components and the risk for neurodegenerative diseases (Morris and Tangney, 2014; Mosconi et al., 2014; Berti et al., 2015). Moreover, long-term consumption of a high-caloric diet is a risk factor that may significantly contribute to the development of neurological disease (Schroeder and Richardson, 2010).

Indeed, an epidemiological study suggested that individuals following diet with high caloric intake have a 1.5 times greater risk of AD than those with low caloric intake (Luchsinger et al., 2002). Interestingly, a number of studies, investigating the association of n−3 polyunsaturated fats (PUFA) or docosahexaenoic acid (DHA) levels (Kitajka et al., 2002) with AD reported reduction of these molecules in *post-mortem* autopsy brain samples (Soderberg et al., 1991; Guan et al., 1994; Han et al., 2001; Green et al., 2007a,b). Major epidemiological surveys shown that dietary n−3 PUFA and/or DHA significantly reduced the risk of developing AD (Kalmijn et al., 1997; Barberger-Gateau et al., 2002; Morris et al., 2003). Moreover, brain DHA levels were positively associated with cognitive and behavioral performance (McCann and Ames, 2005). Indeed, n−3 PUFA supplementation can improve brain function, especially for complex tasks in normal individuals (Fontani et al., 2005).

Also different macro- and micronutrients as B-vitamins and antioxidants can influence brain structure and function (Bourre et al., 2004; Bourre, 2004, 2006a,b; Weih et al., 2007). For instance, polyphenols, in addition to their antioxidant properties, have been reported to exert neuroprotective effects in tg CRND8 mice (Pantano et al., 2016) by directly modulating cellular pathways related to neuronal processes and synaptic

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Abbreviations: AD, Alzheimer disease; wt, wild type; DHA, docosahexaenoic acid; LFPD, low fat-protein diet; PUFAs, polyunsaturated fatty acids; tg, transgenic.

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plasticity (Kawashima et al., 2010). Fatty acids have been implicated in enhancing brain plasticity and cognitive function in healthy adult rodents (Maqsood and Stone, 2016; Wang and Mitchell, 2016; Giles et al., 2016; Weiser et al., 2016) as well as in transgenic mouse models of AD. In fact, enriching the diet of transgenic mice with docosahexaenoic acid (DHA) significantly lowered the synthesis of beta-amyloid peptides and the formation of amyloid plaques, one of the major hallmarks of the disease (Amtul et al., 2011).

The availability of transgenic animal models of AD has permitted investigations of the impact of nutrients and other compounds isolated from foods on AD-related neuropathology, with particular focus on the amyloid-beta peptide production and accumulation in the brain and on behavioral deficits (Kadish et al., 2016; Kothari et al., 2016; Janssen et al., 2016). In a rat AD model caused by injection of amyloid-β ($A\beta_{1-40}$), DHA or eicosapentanoic acid (EPA) were both beneficial agents (Hashimoto et al., 2002, 2006, 2009). Similarly, in various transgenic mouse models supplementation with n−3 PUFA (e.g., DHA) was associated with lower Aβ levels and improved cognition (Green et al., 2007a,b; Fiol-deRoque et al., 2013; Bascoul-Colombo et al., 2016).

The above works indicated that dietary DHA might be beneficial for AD, possibly by alleviating the amyloid pathology.

Different studies have also investigated the role of high-fat diet, high-sugar diet and/or high-cholesterol diet on P-Tau, another pathological hallmark of AD (Refolo et al., 2000; Glöckner et al., 2011; Leboucher et al., 2013). However, the results are conflicting, reporting increased, decreased or unchanged P-tau levels. Therefore, further studies to assess the role of diet on tauopathies are required.

In this work we investigated the impact of a low fat-protein diet on the onset and progress of tauopathy, with a main focus on the phosphorylation of Tau, in the P301L mouse model of tauopathy (Buccarello et al., 2017). In addition, we explored whether a possible sex dimorphic effect may occur. To this purpose, we examined the body weight, food-water intake and survival rate of males and female transgenic P301L-Tau mice fed with standard and low fat-protein diet (LFPD) and wild type mice (wt) fed with standard diet. The 3 months old mice were used for these experiments, and monitored for 12 months. At 15 months of age, mice were tested for behavioral assays to analyze the impact of diet on cognitive/learning and locomotor performance, then sacrificed and processed for immunohistochemical analysis. Together, these results add new perspectives to our understanding on how dietary intake can contribute to AD and Tau-related pathologies, underlying the importance of a correct nutrition in the prevention of neurodegenerative diseases.

MATERIALS AND METHODS

Animals and diets

In this study we used male and female hemizygous P301L-tg mice and age-compatible wild type mice (B6D2F1) of mixed gender as controls. Hemizygous P301L-tg mice carry the mutant form of human tau protein (P301L), which includes four-repeats without amino terminal inserts, and driven by the mouse prion promoter 6 (MoPrP) (Borchelt et al., 1996). Mice originated from Taconic Laboratories, USA, were bred at IRCCS Mario Negri Institute of Pharmacological Research in a Specific Pathogen free (SPF) facility with a regular 12:12 h light/dark cycle (lights on 07:00 a.m.), at a constant room temperature of 22 ± 2 °C, and relative humidity approximately 55 ± 10%. All mice were provisioned with bedding material (hard wood shavings), ad libitum food and water. Animals were housed (n = 4 per group) in standard mouse cages. Until three months of age all animals were fed with standard rodent chow (**Standard diet**: 18% protein and 5% fat, Envigo Lab. 2018S Tekland global diet, http://www.envigo.com/products-services/teklad/laboratory-animal-diets). Three months old animals were then divided into two experimental groups, balanced for body weight and sex. The first group was fed with a standard rodent chow (**Standard diet**: 18% protein and 5% fat, Envigo Lab. 2018S Tekland global diet, http://www.envigo.com/products-services/teklad/laboratory-animal-diets), while the second group was fed with a low fat protein diet (LFPD) (**Low fat protein diet**: 14% protein and 3.5% fat Envigo Lab. 2014S Tekland global diet, http://www.envigo.com/products-services/teklad/laboratory-animal-diets).

Since the animals were bred in a SPF facility, where all materials introduced are sterilized, we used an autoclavable diet manufactured with high quality ingredients and supplemented with additional vitamins to ensure nutritional adequacy after autoclaving.

The **Standard diet** is a fixed formula, autoclavable diet designed to support gestation, lactation, and growth of rodents. This diet does not contain alfalfa, thus lowering the occurrence of natural phytoestrogens. Typical isoflavone concentrations (daidzein + genistein aglycone equivalents) range from 150 to 250 mg/kg. Exclusion of alfalfa reduces chlorophyll, improving optical imaging clarity. Absence of animal protein and fishmeal minimizes the presence of nitrosamines.

The **Low fat protein diet** (LFPD) is a fixed formula, autoclavable diet designed to promote longevity and normal body weight in rodents. This diet does not contain alfalfa or soybean meal, thus minimizing the occurrence of natural phytoestrogens. Typical isoflavone concentrations (daidzein + genistein aglycone equivalents) range from non-detectable to 20 mg/kg. Exclusion of alfalfa reduces chlorophyll, improving optical imaging clarity. Absence of animal protein and fishmeal minimizes the presence of nitrosamines.

Ethics statement

Procedures involving animals and their care were in accordance to the national and international laws and policies (EEC Council Directive 86/609, OJ L 358, 1 Dec.12, 1987; NIH Guide for the Care and use of Laboratory Animals, U.S. National Research Council, 2011). The Mario Negri Institute for Pharmacological Research (IRCCS, Milan, Italy) Animal Care and Use