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Employing Alzheimer Disease Animal Models for Translational Research: Focus on Dietary Components

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Abstract
Background: Translational research needs valid animal models of disease to discover new pathogenetic aspects and treatments. In Alzheimer's disease (AD), transgenic models are of great value for AD research and drug testing. ***Objective:*** It was the aim of this study to analyze the power of dietary polyphenols against neurodegeneration by investigating the effects of oleuropein aglycone (OLE), the main phenol in the extra virgin olive oil (EVOO), a key component of the Mediterranean diet (MD), in a mouse model of amyloid-β deposition. ***Methods:*** TgCRND8 mice (3.5 months old), expressing the mutant KM670/671NL+V717F h-βAPP$_{695}$ transgene, and wild-type (wt) mice were used to study in vivo the effects of an 8-week dietary supplementation with OLE (50 mg/kg of diet) [Grossi et al: PLoS One 2013;8:e71702], following the European Communities Council Directive 86/609 (DL 116/92) and National Guidelines (permit number: 283/2012-B). ***Results:*** OLE administration ameliorates memory dysfunction, raises a significant autophagic response in the cortex and promotes the proliferation of newborn cells in the subgranular zone of the dentate gyrus of the hippocampus. ***Conclusions:*** Our findings support the beneficial effects of EVOO and highlight the possibility that continuous intake of high doses of OLE, both as a nutraceutical or as a food integrator, may prevent/delay the appearance of AD and reduce the severity of its symptoms.

Alzheimer's disease (AD) is the most common neurodegenerative disorder affecting a large proportion of aged people in the developed countries. It is characterized by massive neuronal cell and synapse loss at specific sites, by extracellular deposition of amyloid-β (Aβ) peptide into senile plaques as well as by intracellular accumulation of tau proteins as neurofibrillary tangles (NFTs) and neuropil threads. Both Aβ plaques and NFTs are considered distinctive histopathological features of AD, and their presence is mandatory for post mortem diagnosis. The neurodegenerative process in AD matches reactive proliferation of glial cells. Although neuronal loss, including loss of cholinergic neurons of the basal forebrain, is not among the diagnostic criteria, it is important for drug development against AD and has been replicated in disease models. Electrolytic or toxin destruction of the choliner-

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gic system of the basal forebrain results in the loss of choline acetyltransferase-positive neurons and behavioral dysfunctions [1] mimicking those occurring in AD and those that are amenable to treatments including the use of cholinesterase inhibitors.

AD experimental models could mimic individual or multiple alterations found in AD; to date, a single model replicating all the alterations observed in AD does not exist. The best model is the aged monkey, in which cognitive assessments can be made that approximate cognitive processes impaired in human AD. Aged rodents do not spontaneously develop Aβ plaques and NFTs; Aβ features have been reproduced using intracerebral injection/infusion of various Aβ peptides [2].

Developed transgenic mouse models, carrying single or combined mutations associated with familial AD [3], knockout models, *Drosophila, Caenorhabditis elegans* and *Zebrafish* models [4, 5], and the transgenic rat model [6] have provided invaluable contributions to the knowledge of molecular and cellular mechanisms of AD, paving the way to possible therapeutic strategies.

Drug Targets in AD Models

The goal of AD treatments depends on the severity of the pathology. In patients with mild to moderate disease, the goal is to improve or maintain baseline performance by administration of disease-modifying drugs. As disease progresses, treatment aims at slowing the rate of decline in performance, mainly through symptomatic therapies that improve cognitive and behavioral deficits. Strategies for evaluating the efficacy of neuroprotective or disease-modifying drugs typically involve the measurement of markers of disease progression, such as Aβ levels, the number and morphology of Aβ plaques and tangle formation in animal models that develop these features over time, together with the development of molecules able to act against the appearance of toxic oligomeric intermediates. Recently developed neuroimaging techniques are promising the evaluation of disease progression at the histopathological level in human subjects [7].

In animal models of AD, amyloid load results from the disturbance of complex equilibria between Aβ generation/deposition and its clearance, where autophagy appears to play a key role [8, 9], together with the activation of glial cells. Autophagy protects neurons against Aβ-induced cytotoxicity suggesting its possible role in Aβ clearance; moreover, efficient autophagy seems to protect against neurodegeneration and to increase longevity. The involvement of autophagy dysfunction in many pathological conditions has raised great interest in identifying drugs that can be used to manipulate autophagy for therapeutic purposes, particularly against neurodegeneration.

Mediterranean Diet

In recent years, several epidemiological data have underscored a possible protective role of nutrition, mostly when it includes a significant amount of natural phenols. A number of nutrients found in the Mediterranean diet (MD), including red wine polyphenol resveratrol [10], have been associated with protection against cognitive decline, dementia and AD in preclinical or epidemiological studies. Mounting evidence supports that greater adherence to MD is associated with a slower rate of decline in the Mini Mental Status Exam, a reduced risk of conversion from mild cognitive impairment to AD [11] and with AD risk reduction. Studies in rodents suggest that diet supplementation with phenol-rich components of MD, such as red wine and extra virgin olive oil (EVOO), improves learning and behavioral deficits associated with aging and disease [12, 13]. Consumption of red wine attenuates AD-like pathology and improves learning and memory in Tg2576 mice, and phenols and secoiridoids found in EVOO have been considered potentially responsible for the beneficial effect of MD [10, 11].

OLE Protects TgCRND8 Mice against AD-Like Pathology

We have recently investigated the beneficial effects of an 8-week dietary supplementation with OLE (50 mg/kg of diet), the main phenol found in EVOO, in TgCRND8 (tg) mice at initial (3.5 months) and intermediate (6 months) stages of Aβ deposition [14]. Tg mice develop a pattern of Aβ deposition recalling several aspects of human AD. Small-sized Aβ42-immunopositive plaques appear in various brain areas, including the cortex and hippocampus, by the age of 3 months. As a function of age, they become small-medium to big in size and acquire a compact core, and accumulate and reach the maximum roughly by 7–8 months of age [15]. OLE administration resulted in a remarkable improvement in animal behavior compared to normally fed littermates, with scores reaching those displayed by age-matched controls. Improved behavior of tg mice at both ages (3.5 and 6 months

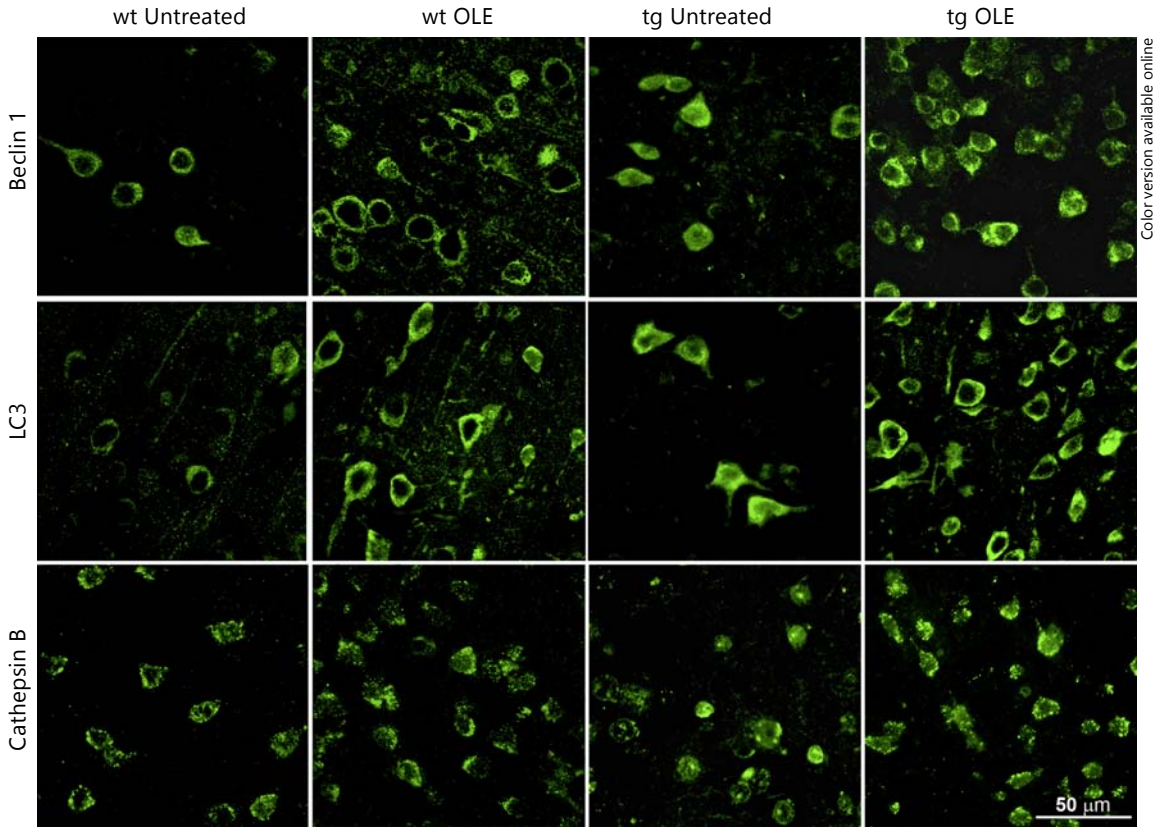

Fig. 1. Immunoreactivity of autophagosome (beclin 1 and LC3) and lysosome (cathepsin B) markers in the cortex of 3.5-month-old OLE-fed and untreated tg and wt mice. Note the strong immunoreactivity in the OLE-fed tg and wt mice [for procedures, see ref. 14].

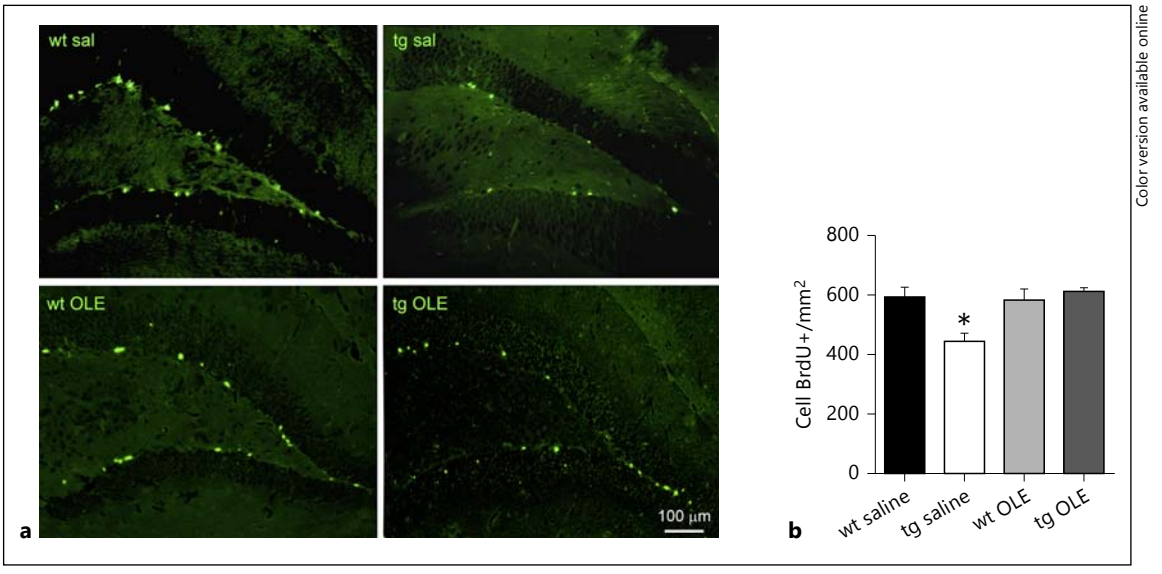

Fig. 2. Cell proliferation in the subgranular zone and effect of OLE diet. **a** BrdU-positive cells of the neurogenic dentate gyrus area in 3.5-month-old OLE-fed and untreated tg and wt mice. sal = Saline. **b** The number of BrdU+ cells is significantly lower in the untreated tg than in the OLE-fed tg mice (* $p < 0.01$, one-way ANOVA plus Newman-Keuls post hoc test; n = 5/group) [for procedures, see ref. 16].

old) was accompanied by a significant reduction in Aβ40/Aβ42 tissue levels and Aβ plaque size and compactness, with less core deposits in the older mice. OLE administration not only prevented amyloid deposition but also disaggregated preformed plaques. Amelioration of cognitive function and neuropathology of young/middle-aged tg animals following OLE administration matched a remarkable induction of autophagy in the brain cortex. A strong punctuate immunoreactivity and high levels of autophagosome-lysosome markers (beclin 1, LC3, cathepsin B) were detected in the cortex of OLE-fed tg mice, as compared to untreated mice (fig. 1) [14]. Moreover, OLE administration significantly increased the proliferation of newborn cells in the subgranular zone of the hippocampus in 3.5-month-old tg mice (fig. 2). Altogether, our findings demonstrate that OLE treatment of tg mice strongly induces autophagy in the cortex and significantly stimulates hippocampal neurogenesis. These concomitant responses suggest their cooperation in the final protective effect, supporting the possibility that dietary supplementation with OLE may prevent/delay the occurrence of AD and reduce the severity of its symptoms.

References

1 Casamenti F, Di Patre PL, Bartolini L, et al: Unilateral and bilateral nucleus basalis lesions: differences in neurochemical and behavioral recovery. Neuroscience 1988;24:209–215.
2 Giovannelli L, Casamenti F, Scali C, et al: Differential effects of amyloid peptides beta-(1–40) and beta-(25–35) injections into the rat nucleus basalis. Neuroscience 1995;66:781–792.
3 LaFerla FM, Green KN: Animal models of Alzheimer disease. Cold Spring Harb Perspect Med 2012;2:a006320.
4 Rockenstein E, Crews L, Masliah E, et al: Transgenic animal models of neurodegenerative diseases and their application to treatment development. Adv Drug Deliv Rev 2007;59:1093–1102.
5 Paquet D, Schmid B, Haass C, et al: Transgenic *Zebrafish* as a novel animal model to study tauopathies and other neurodegenerative disorders in vivo. Neurodegener Dis 2010;7:99–102.
6 Cohen RM, Rezai-Zadeh K, Weitz TM, et al: A transgenic Alzheimer rat with plaques, tau pathology, behavioral impairment, oligomeric Aβ, and frank neuronal loss. J Neurosci 2013;33:6245–6256.
7 Zhang L, Chang RC, Chu LW, et al: Current neuroimaging techniques in Alzheimer's disease and applications in animal models. Am J Nucl Med Mol Imaging 2012;2:386–404.
8 Rubinsztein DC, Marino G, Kroemer G, et al: Autophagy and aging. Cell 2011;146:682–695.
9 Mizushima N, Komatsu M: Autophagy: renovation of cells and tissues. Cell 2011;147:728–741.
10 Ho L, Chen LH, Wang J, et al: Heterogeneity in red wine polyphenolic contents differentially influences Alzheimer's Disease-type neuropathology and cognitive deterioration. J Alzheimers Dis 2009;16:59–72.
11 Feart C, Samieri C, Rondeau V, et al: Adherence to a Mediterranean diet, cognitive decline, and risk of dementia. JAMA 2009;302:638–648.
12 Pitozzi V, Jacomelli M, Zaid M, et al: Effects of dietary extra-virgin olive oil on behaviour and brain biochemical parameters in ageing rats. Br J Nutr 2010;103:1674–1683.
13 Karuppagounder SS, Pinto JT, Xu H, et al: Dietary supplementation with resveratrol reduces plaque pathology in a transgenic model of Alzheimer's disease. Neurochem Int 2009;54:111–118.
14 Grossi C, Rigacci S, Ambrosini S, et al: The polyphenol oleuropein aglycone protects TgCRND8 mice against Aβ plaque pathology. PLoS One 2013;8:e71702.
15 Bellucci A, Rosi MC, Grossi C, et al: Abnormal processing of tau in the brain of aged TgCRND8 mice. Neurobiol Dis 2007;27:328–338.
16 Fiorentini A, Rosi MC, Grossi C, et al: Lithium improves hippocampal neurogenesis, neuropathology and cognitive functions in APP mutant mice. PLoS One 2010;5:e14382.