Plant Extracts Stimulate the Autophagy-Lysosomal Pathway and Improve Synaptic Markers in a Brain Slice Model of Age-Related Protein Accumulation Stress

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Abstract

Removal of old and damaged proteins becomes less efficient with age and causes gradual protein accumulation pathology, leading to synaptic compromise, cognitive decline, and contributing as the primary risk factor of dementia. The positive modulation of the lysosomal protease cathepsin B (CatB) appears to reduce the protein accumulation and improve synaptic integrity. Interestingly, the protease CatB was suggested as a mediator of the effects of exercise on cognition in human, primates, and mouse. Besides, people with a healthy diet have lower levels of protein accumulation in the brain and 40% less chance of dementia. In this study, using slice cultures of rat hippocampus plant extracts applied daily for three days, followed by an assessment for changes in synaptic markers and components of the autophagy-lysosomal pathway as compared to vehicle-treated samples. American ginseng (P. quinquefolius) and bacopa (B. monnieri) extracts markedly enhanced the lysosomal protease cathepsin B (CatB). Whereas only brain tissue treated with American ginseng exhibited a correlation between CatB levels and improved measures of the synaptic protein GluR1. The extract-infused hippocampal slice cultures were also treated with the lysosomal inhibitor chloroquine (CQN) and tested for protection against protein accumulation stress-induced synaptic compromise. American ginseng-treated slices were less prone to synaptic decline and protein ubiquitination due to CQN-mediated protein accumulation stress. Therefore, the enhanced autophagy-lysosomal pathway protected brain synapses in a model of age-related deficiency in protein clearance activity, suggesting a need for additional studies to test for benefits in aged animals with cognitive impairment.

The incidence of age-related neurodegenerative disorders is the greatest future global health care challenges since life expectancy are rinse-up in developed and economic emergent countries. Aging is the major risk factor for the development of age-related diseases, e.g., Alzheimer's disease (AD). Currently, 5.8 million Americans are living with AD, and by 2050 this number is projected to rise to 14 million. The U.S. authorities expect that AD and related dementias will cost 290 billion dollars to the government in 2019 (Alzheimer Association, Facts, and Figures, 2019). Thus, the society seeks to find an efficient method for preventing brain aging-induced cognitive decline and related dementias.

Brain aging causes gradual protein accumulation pathology as clearance systems depreciate, leading to synaptic compromise, cognitive decline, and contributing as the primary risk factor of dementia. Removal of old and damaged proteins occurs through the proteasome and autophagy-lysosomal systems, and becomes less efficient with age altering the balance between protein synthesis and protein clearance (see Bahr, 2009; Li & Li, 2011; Nixon and Yang, 2012; Bahr et al., 2012). In experimental models of age-

related protein pathology by: unbalance protein clearance systems (Farizatto et al., 2017), self-aggregation of amyloid-β oligomers (Wisniewski et al., 2011), or using a transgenic mice expressing different levels of amyloid-β pathology (Butler et al., 2011), the projected protein accumulation was linked to secondary synaptic damage. Interestingly, the positive modulation of the lysosomal protease cathepsin B (CatB) appears to reduce the protein accumulation and improve synaptic integrity (Butler et al., 2011; Farizatto et al., 2017). Recently, Moon and colleagues (2016) suggested the CatB as a mediator of effects of exercise on cognition, due: i) elevated levels of CatB in muscle and plasma of runners; ii) the absence of hippocampal neurogenesis and spatial memory in CatB knockout mice; and iii) the positive CatB levels correlation with fitness and hippocampus-dependent memory function. Taken together, these data suggest that positive modulation of protein clearance leads clear the protein accumulation appears to be a potential therapeutic avenue against neurodegenerative diseases.

Interestingly, Scarmeas and colleagues (2006) showed that people that attended a healthy diet have a 40% less chance of dementia than people that consume dairy products and meat. The menus based on whole grains, fruit, vegetables, fish, and olive oil prone to decrease the brain atrophy and lower levels of protein accumulation in the brain (Berti et al., 2018; Moconi et al., 2018). Indeed, poor nutrition is thought to influence cognitive aging, and a growing number of studies point to natural products and a healthy diet as avenues for promoting brain health.

Geographical and epidemiological studies pinpoint that a diet rich in antioxidants and plant-based foods (i.e., Mediterranean diets and traditional Japanese diets) promote longevity and healthy cognitive aging (Trichopoulou & Vasilopoulou, 2000; Willcox et al., 2007). Additionally, previous reports have found that plant-based dietary intervention facilitates cognitive enhancement in both aged rodent models and human subjects (see Joseph et al., 2009; see Spencer et al., 2009). However, a definitive therapeutic target to elicit improved cognitive aging remains a topic to be debated. Thus, the Almeida and colleagues (in preparation) aim were to screen a group of plant extracts for the ability to amplify the brain's autophagy-lysosomal protein clearance pathway and to determine if such amplification reduces the synaptic decline in a brain slice model of protein accumulation stress.

Using slice cultures of rat hippocampus, a brain region vulnerable to Alzheimer's disease and aging, plant extracts (1-500 μg/ml) were applied daily for three days, followed by an assessment for changes in synaptic markers and components of the autophagy-lysosomal pathway as compared to vehicle-treated samples. The extract-infused hippocampal slice cultures were also treated with the lysosomal inhibitor chloroquine (CQN) and tested for protection against protein accumulation stress-induced synaptic compromise. American ginseng (P. quinquefolius) and bacopa (B. monnieri) extracts markedly enhanced

the lysosomal protease cathepsin B (CatB). They both produced a nearly 4-fold increase in the 30-kDa active form of CatB (CatB-30), whereas only brain tissue treated with American ginseng exhibited a correlation between CatB levels and improved measures of the synaptic protein GluR1. Small increases in CatB-30 were produced by extracts from Panax ginseng and wild blueberry (V. myrtillus). Also a primary outcome, American ginseng-treated slices were less prone to synaptic decline due to CQN-mediated protein accumulation stress. In conclusion, plant extracts differentially enrich CatB in hippocampal tissue in a manner that positively influences synaptic integrity. Enhancing the autophagy-lysosomal pathway protected brain synapses in a model of age-related deficiency in protein clearance activity, suggesting a need for additional studies to test for benefits in aged animals with cognitive impairment.

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