Plant extracts stimulate the autophagy-lysosomal protein clearance pathway and improve brain synapse markers in a brain tissue model of age-related protein accumulation stress

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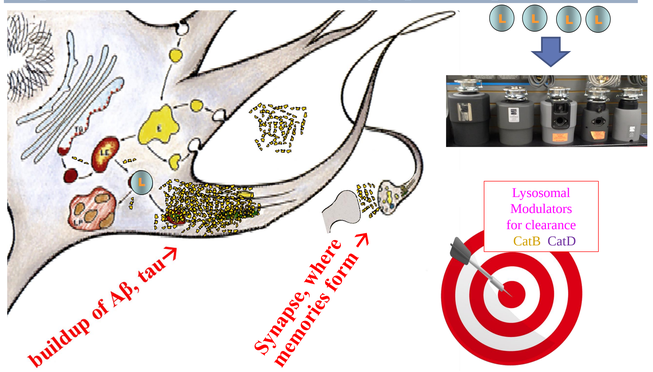
Abstract

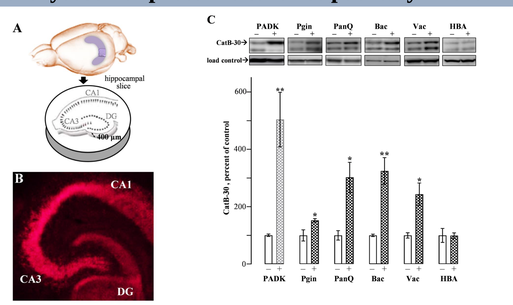
Brain aging causes gradual protein accumulation pathology as clearance systems depreciate, leading to synaptic compromise, cognitive decline, and contributing as the primary risk factor of dementia. Removal of old and damaged proteins becomes less efficient with age, Poor nutrition is thought to influence cognitive aging and a growing number of studies point to natural products and a healthy diet as avenues for promoting brain health. The aim of this study was 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 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 3 days, followed by 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. 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.

1. Age-related disorders (e.g. Alzheimer's disease) exhibit protein accumulation linked to synaptic compromise



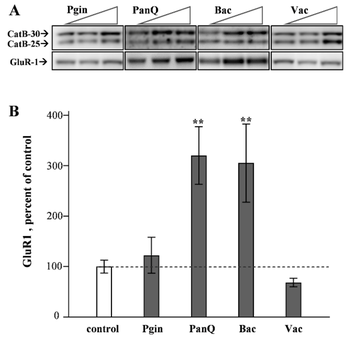
Protein accumulation pathology leading to synaptic compromise. Brain aging causes the protein clearance systems ineffective. Enhance the autophagy-lysosomal pathway label as a therapeutic avenue can clear the protein accumulation and recovery the synaptic integrity.

2. Certain natural extracts enhance cathepsin B (CatB) of the lysosomal protein clearance pathway in brain slices



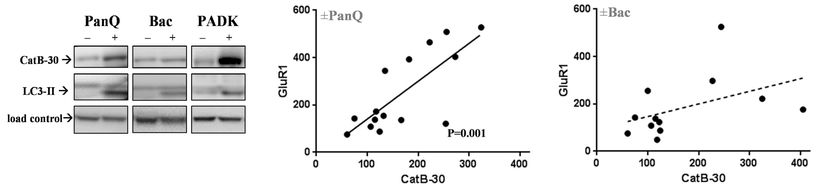
Natural extracts and polyphenolic compounds enhance the lysosomal protease cathepsin B in hippocampal slice cultures. Rat hippocampal tissue from postnatal was removed and transverse slices with 400 µm thickness were maintained on Biopore inserts 2-3 weeks (A), exhibiting Nissl-fluorescence stained neuronal subfields (B, view-field width: 1.5 mm). CA, Cornu Ammonis; DG, dentate gyrus. Organotypic hippocampal slice treated with either natural extracts or polyphenolic compound (PADK, HBA) were harvest and equal content of protein were assessed by immunoblot against CatB-30 antibody (C). Immunoreactivity levels of CatB-30 were normalized to their respective control data (percent of control; mean±SEM). Unpaired t-tests: *p<0.05; **P≤0.01.

3. Extracts of American Ginseng (PanQ) and Bacopa (Bac) enhance cathepsin B and improve synaptic markers in brain slices cultures



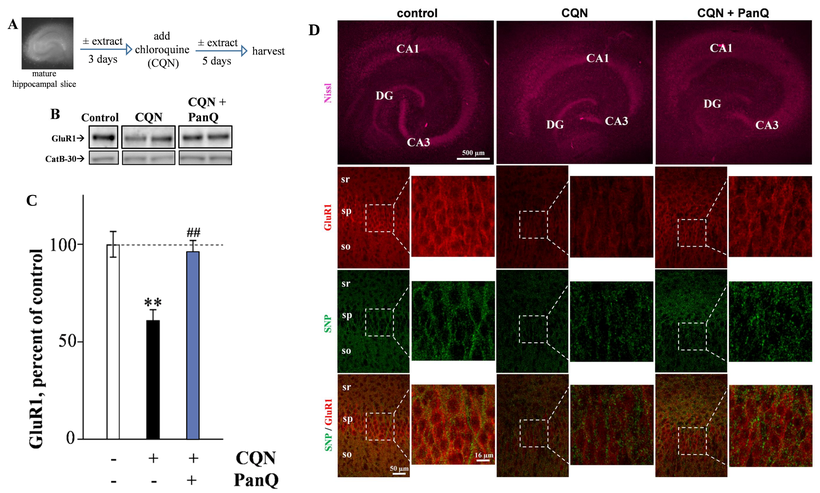
Selective natural extracts enhance both the cathepsin B and synaptic marker in hippocampal slice cultures. Immunoblotted analysis assessed hippocampal slice cultured treated with natural rising dose of extracts that displayed enhancement on Cathepsin B levels was also immunoblot against AMPA-receptor subunit GluR1 (A). Pgin: 20-100 µg/ml, PanQ: 100-500 µg/ml, BacM: 55-210 µg/ml, and Vcor: 8-100 µg/ml. GluR1 immunoreactivities was measured by integrated optical density and showed as mean percent control ± SEM (B). Unpaired t-tests: **p<0.01.

4. American Ginseng is the better enhancer of autophagy-lysosomal components, and this enhancement correlates with synapse integrity



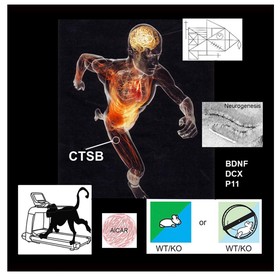
Selective natural extracts enhance the cathepsin B and a marker for mature isoform of autophagy vesicle. Rat hippocampal slice cultures treated with selective natural extracts (PanQ and Bac) or cathepsin B positive modulator (PADK) were harvest and equal content of protein were assessed by immunoblot against cathepsin B (CatB-30), LC3-II, GluR-1 and synaptophysin (SNP). *Panax quinquefolius* (PanQ) exhibited a correlation between CatB levels and improved measures of the synaptic protein GluR1. The immunoreactivity levels of the synaptic marker GluR1 and CatB across individual slice samples treated with or without PanQ (A) were plotted again each other and linear regression result and tested to correlation analysis. The Pearson' correlation analysis displayed for PanQ: R^2=0.5738; p=0.001; and Bac: R^2=0.193) Dotted lane are showing non significance correlate was found.

5. American Ginseng also protects against synaptopathology in a model of Alzheimer-type protein accumulation stress



Mature hippocampal slice cultures were pre-treated with 100 µg/ml PanQ 3 days alongside vehicle-treated slices followed by the lysosomal inhibitor chloroquine (CQN, 60 µM) with PanQ or vehicle for 5 days then harvested (A), and assessed by immunoblot for GluR1 and CatB-30 (B). The GluR-1 immunoreactivies measured were normalized to controls, and percent of control mean ± SEM are shown (C). Unpaired t-test: **p=0.0012 (control vs CQN); ## p=0.0014 (CQN vs CQN + PanQ). Hippocampal slices cultured pre-treated with PanQ by CQN insult in presence or absence of PanQ were fixed 24 h and assessed by immunostaining for Nissl fluorescent stain, anti-GluR1, synaptophysin. sp, stratum pyramidale; sg, stratum granulosum; size bar: 50 µm.

6. Exercise is the only avenue shown to reduce the risk of Alzheimer's: appears to involve cathepsin B



Reduce your RISK for AD!

The CatB enhancement that we studied also occurs in exercise in humans, and the CatB levels correlate with improved memory function.

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

National Institute on Aging, and German Center Neurodegenerative Diseases
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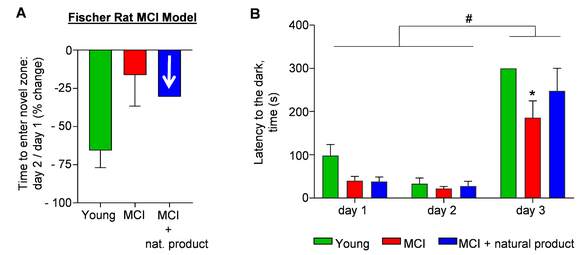
A healthy diet can reduce the likelihood of getting Alzheimer's by 40%

- Attending a heath diet have a 40% less chance of dementia than people that consume dairy products and meat (Scarmeas et al., 2006).
- Diets based on whole grains, fruit and 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).

7. Preliminary studies: *in vivo* model of Mild Cognitive Impairment (pre-AD)



Mild Cognitive Impairment (MCI) cause learning and memory issues in middle-aged female rats, and selective natural products seems to prevent the cognitive decline.

Plants extract through CatB to reduce the risk of AD

- Plant extracts differentially enrich CatB in a brain region important to memory, that in some cases leads positively influences synaptic integrity.
- Enhancing the autophagy-lysosomal pathway protected brain synapses in a model of age-related deficiency in protein clearance that can lead to AD risk.
- Health diet, physical activity and natural products may explain the lower incidence of Alzheimer in certain areas in the World.

Acknowledgements & References

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