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Short-Chain Fatty Acids Analysis in Brain by GC/MS to Determine the Effect of Bioactive Food in a Mouse Model of Alzheimer's Disease

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Introduction

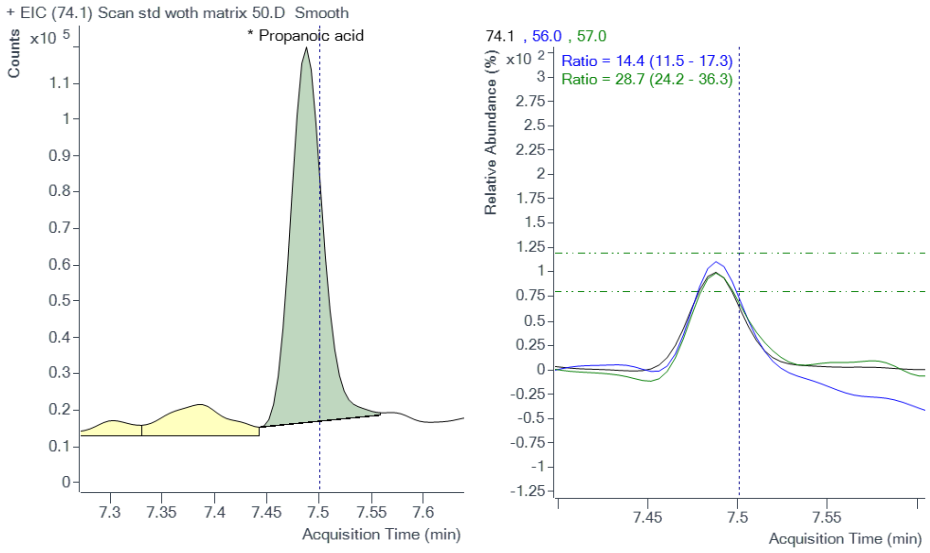
Recent investigations have demonstrated an important role of gut microbiota (GM) in the pathogenesis of Alzheimer's disease (AD). GM modulates a host's health and disease manifestation by production of several substances, including lipopolysaccharides (LPS) and short-chain fatty acids (SCFAs), among others[1]. Diet can modify the composition and diversity of GM, and ingestion of a healthy diet has been suggested to lower the risk of developing AD. Studies have shown that bioactive food (BF) ingestion can abate neuroinflammation and oxidative stress and improve cognition in obese rats, effects associated with GM composition. Therefore, BF can impact the gut-brain axis and improved behavior. Therefore, the neuroprotective effects of BF may be mediated, in part, by modulation of GM and the release of neurotoxic substances that alter brain function. Alzheimer's disease (AD) is a degenerative brain disease and the most common cause of dementia. In AD, aggregation of amyloid- (A) protein and hyperphosphorylation of tau represents the major pathological hallmarks[1]. On the one hand, misfolded and aggregated proteins are recognized by astroglia and microglia cells and trigger an innate immune response, characterized by the release of inflammatory cytokines and neuroinflammation, which contribute to disease progression and severity . On the other hand, it has been postulated that soluble A may cause neuronal membrane damage, producing reactive oxygen and nitrogen species[2]. This oxidative damage would alter synaptic membrane structure, causing alterations in dendritic spines with a subsequent cognitive decline[3]. In addition, data obtained from AD research has shown that hyperactivity of hippocampal neurons precedes amyloid plaque formation[4]. Thus, AD pathogenesis is a multifactorial disease that conveys different mechanistic pathways[1]. Diet can modify the composition and diversity of GM, and ingestion of a healthy diet has been suggested to lower the risk to develop AD. In the present study, SCFA content in feces and brain samples were analyzed by gas chromatography mass spectrometry (GC/MS) to demonstrate that BF ingestion diminished the main pathological markers of AD.

Experimental

Female 3×Tg-AD transgenic mice (TG) (RRID:MMRRC 034830-JAX) harboring the APPSWE and TauP301L transgenes on a PS1M146V knockin background (homozygous mutant APPSWE, PS1M146V, and TauP301L), and female wild-type (WT) B6129SF1/J (RRID:IMSR JAX:101043) from same genetic background as PS1M146V knock-in mice, but harboring the endogenous wild-type mouse PS1) (both Jackson Laboratory, Bar Harbor, ME,USA) were used for the study. All mice were housed with access to food (Purina RodentChow5001) and water *ad libitum*, and under optimal vivarium conditions (12 h/12 h light–dark cycle, 20°C, and 40–50% relative humidity). Cognitive assessment, Immunohisto-chemistry, immunofluorescence, Western blot, fecal microbiota analysis, and analysis of short-chain fatty acids in feces and brain were performed.

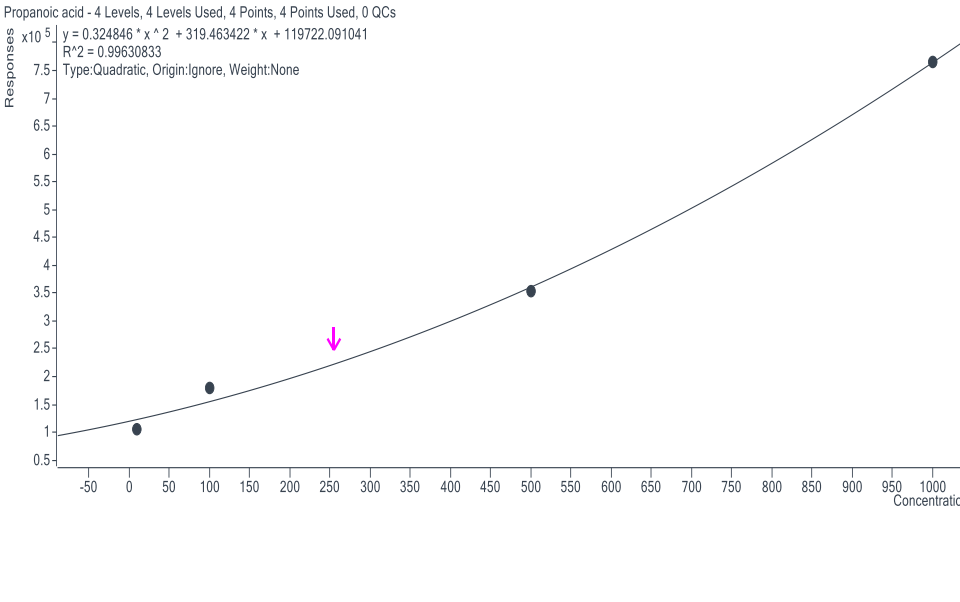
To evaluate the levels of propionic acid in brain, we used a GC/MSD - an Agilent Intuvo 9000 gas chromatography system coupled to an Agilent 5977B mass spectrometric detector (MSD, Agilent Technologies, Santa Clara, CA). No sample preparation was required as a thermal separation probe accessory was used for introduction of the sample into the GC/MSD. Samples were weighed and loaded into the vial for propionic acid extraction at the inlet, followed by the standard GC/MS analysis. Propionic acid was separated using an Agilent column (part number 122-7033UI-INT: Serial number US16460202), phase DB-WAX UI (Dimensions 30m×250m×0.5m). A sample of 0.4 µL was injected in split mode with a ratio of 20:1, and the solvent delay time was set to 6 min. Helium was used as a carrier gas at a constant flow rate of 1 mL/min. The initial oven temperature was held at 60°C for 0.05 min, ramped to 250◦C at a rate of 900°C/min, and finally held at this temperature for 3 min. Data analysis was performed using MassHunter Quantitative software. (Agilent Technologies, Santa Clara, CA,USA).

Results and Discussion

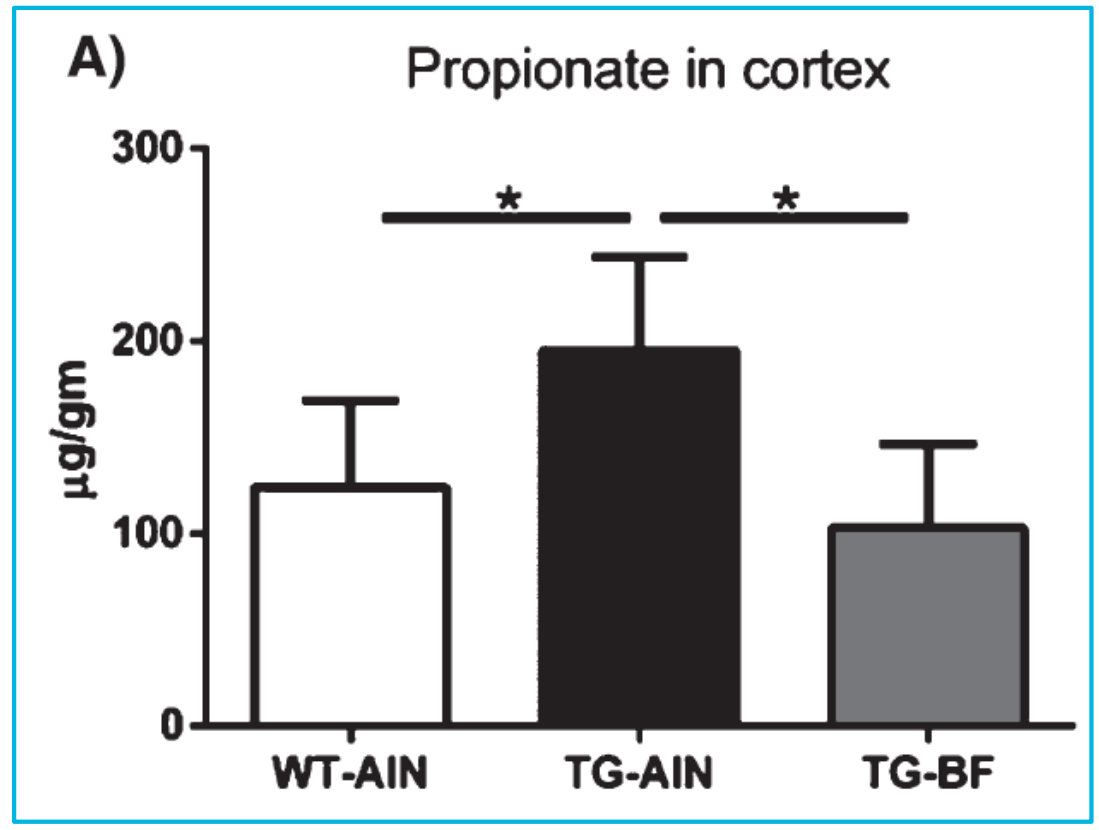


Quantification and qualifiers signals for propionic acid

The standard was injected with matrix and 3 ions were selected - one was used as a quantifier ion and the others as qualifiers. Retention time was used to identify the propionic acid.



Propionic acid calibration curve from 10 µM to 1000 µM



Propionate concentration in cortex (bacterial products in brain and plasma samples)

Propionate levels in prefrontal cortex were higher in transgenic mouse with ingestion standard diet TG-AIN compared to wild-type WT-AIN mice ($p < 0.05$) and transgenic mouse with ingestion of bioactive food (BF) reduced those levels ($p < 0.05$).

We observed that TG groups had significantly higher body weight compared to WT mice[1]. However, BF ingestion by TG mice did not modify food intake, nor weight gain with respect to the control group. Body weight changes in TG mice have been associated with glucose intolerance.

Spatial and working memory were assessed using a T-maze test[1]. Percentage of spontaneous alterations decreased in TG-AIN compared to WTAIN mice, but BF ingestion for 7 months significantly improved cognition in TG-BF mice.

Results and Discussion

Propionate level and FFAR3 in brain

The PFC is an area related to T-maze working memory performance, and we have observed working memory impairment in TG mice[1]. Therefore, we evaluated the levels of propionate in this brain region. Propionate content was larger in TG-AIN mice compared to WT-AIN and TG-BF mice in PFC. We also quantified SCFA receptor FFAR3/GPR41 in the cortex. FFAR3 has a high affinity for propionate and was more abundant in TG-AIN mice compared to WT-AIN mice, whereas BF ingestion decreased those values in TG mice.

In this study, we demonstrated that the ingestion of BF diminished the main pathological markers of AD, amyloid aggregates and hyperphosphorylation of tau, in TG female mice[1]. BF also diminished neuroinflammation and synaptic and metabolic alterations, and improved working memory in TG-BF compared to TG-AIN mice, effects associated with the reduction in pro-inflammatory gut bacteria and their products. We used female 3xTg-AD mice as recent evidence indicates that women are at greater risk for developing the disease[4], and female TG mice have more severe pathology compared to males. Therefore, we aimed to evaluate the effects of BF on the more vulnerable gender. We concluded seven months of dietary treatment when female TG were 9 months old, an age where they show neurogenic and neuroplastic deficits resulting in cognitive decline[1]. However, at 9 months of age, the pathological hallmarks and cognitive impairment were moderately present in our 3xTg-AD strain. Therefore, we assessed other markers which appear since early stages of the disease.

Conclusions

Cognitive assessment, immunohistochemistry, immunofluorescence, Western blot, fecal microbiota analysis, and short-chain fatty acids analysis in feces and brain, indicate that pro-inflammatory bacteria released substances cause, on the one hand, LPS-induced immune activation of glia cells[1]. On the other hand, uptake of propionate by astrocytes as energy source induces SIRT1 increase; both events, can be associated with an impaired Glu-Gln shuttle, resulting in synaptic dysfunction and memory impairments in TG mice. All those alterations were restored in TG mice after ingestion of BF during 7 months. Based on the present data, we propose that a dietary intervention at an early stage of Alzheimer's disease pathology is an effective strategy to abate GM dysbiosis with pro-inflammatory profile, associated with amyloid pathology, metabolic, and synaptic alteration, resulting in a better cognitive outcome.

References

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