Phytochemicals of Watercress Juice: Prevention of Amyloid Beta Peptide Aggregation

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Background

The most prevalent age-related neurodegenerative condition is Alzheimer's disease (AD), commonly known as senile dementia. In addition to gradual memory loss and cognitive dysfunction, (AD) results in personality changes, physical skill loss, and mortality. According to histology, intracellular and extracellular neurofibrillary tangles are the primary features of (AD). These tangles cause the death of neurons and synapses, which results in gross atrophy of the brain. Amyloid beta-peptide (Aβ) production and deposition are frequently considered to be important contributors to (AD) pathogenesis. The peptide (Aβ), which is 38–43 amino acids long, is produced by sequentially cleaving of the amyloid precursor protein (APP). The integral membrane protein is 695-771 amino acids long and is present in a variety of tissues and organs. According to previous HPLC studies, Watercress juice contained derivatives of kaempferol. Kaempferol (KMP) has been depicted as a revolutionary attribute in overall human health ranging from anti-cancerous to anti-inflammatory properties. Here we show a novel approach of exploring Kaempferol and its derivatives (Glucoside and Rutinoside) impact and effects on the aggregation behavior of amyloid-β peptide.

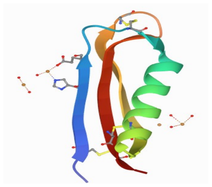

Figure 1. (Aβ) Peptide.

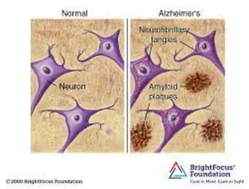

Figure 2. Amyloid β-Plaques

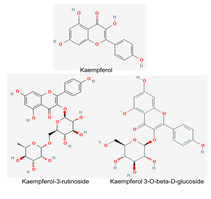

Figure 3. Structure of Kaempferol and derivatives.

Objectives and Approaches

Hypothesis: We hypothesize that Kaempferol and its derivatives (Rutinoside and Glucoside) will affect the aggregation behavior of Aβ.

- **Objective:** Characterize the aggregation behavior of (Aβ) when exposed to Kaempferol and it's two derivatives in solution.
- **The approaches taken were:**
  - CD spectroscopic studies of the Aβ peptide in separate solutions at 37° C with KMP and its two derivatives
  - Fluorescence spectroscopic studies of the peptide using the amino acid 7-azatryptophan (7AW) as an intrinsic probe.
  - Atomic Force Microscopy (AFM) is being performed simultaneously.

Experimental Techniques

- **Fluorescence Spectroscopy:** PerkinElmer 6500 fluorimeter. Excitation and emission slit widths were 10/10 nm unless specified.
- **CD Spectroscopy:** Jasco J-815 CD Spectrometer

Materials and Methods

- **Amyloid Beta** (Aβ) peptides 1-42-7-AW (pepMic, MW: 4701.22, Purity: >95%, Quantity: 1.0mg), and 1-42 human (pepMic, MW: 4514.07, Purity: >95%, Quantity: 1.0mg)
- 10 mM **sodium hydroxide** (NaOH) solution (Sigma Aldrich)
- **Kaempferol** (Sigma Aldrich),
- **Kaempferol 3-rutinoside** (Sigma Aldrich)
- **Kaempferol 3-O-beta-glucoside** (Sigma Aldrich).

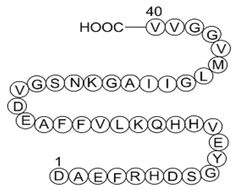

Figure 4. (Aβ) Peptide (I-40)

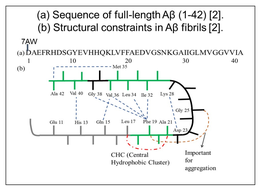

Figure 5. Modified (Aβ) Peptide (I-42)

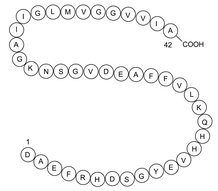

Figure 6. (Aβ) Peptide (I-42)

CD Spectroscopy of AB in solution

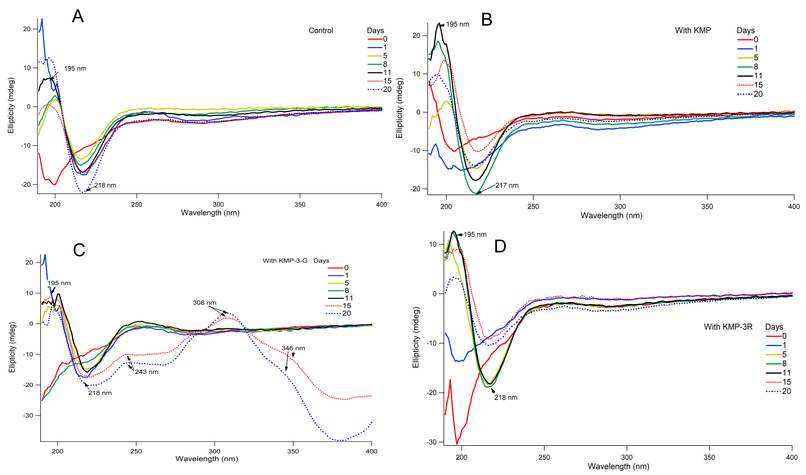

Figure 7. The secondary structures of the control and KMP/derv. treated Aβ peptides are shown in here. Studies are conducted for 20 days at 37°C.

Observations:

1. Compared to the control, in the Aβ samples containing kaempferol and it's Rutin and Glucose derivates, Beta-Sheet formation was delayed.
2. Samples containing the glucose derivate, had a lower intensity for the second (positive) peak.
3. CD spectra of Day 15 and 20 of the Aβ peptide with kaempferol-glucose derivative showed an induced-CD indicating a modification in the peptide backbone by the derivative.

Fluorescence Spectroscopy of AB in solution

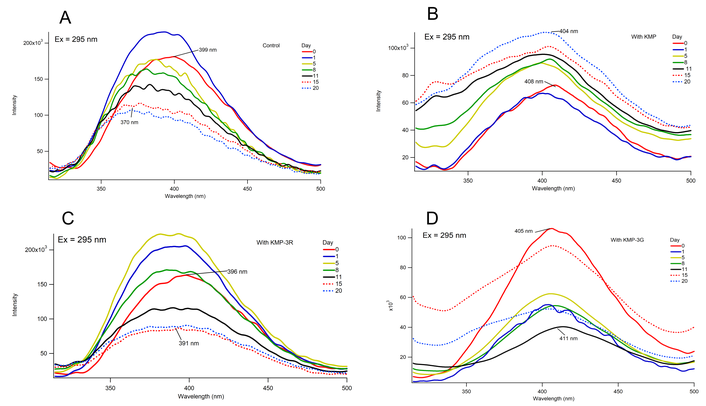

Figure 8. Fluorescence spectroscopic studies of the control and KMP/derv. treated 7AW-Aβ peptides are shown in here. Studies are conducted for 20 days at 37°C.

Observations:

1. Emission spectra displayed a blue shift among most of the samples, which suggests that aggregation is occurring, but at a slower rate in the presence of derivatives, compared to the control.

Conclusions

Kaempferol and its derivatives appear to delay the β-sheet formation of the Amyloid β- Peptide. Compared to the other solutions, the sample containing rutin derivative delayed β-sheet formation greater than Kaempferol and Glucose derivative. Since the emission spectra displayed a blue shift among all samples, it suggests that the peptide is aggregating.

Future Studies

1. Study the aggregation behavior of the Aβ samples with watercress juice and extract at 37°C.
2. Compare the results with the findings from Kaempferol and the derivatives.
3. Investigate AFM images and explore if the AFM images corroborate with the spectroscopy studies.
4. Repeat the experiment with sections of the Aβ peptide to find out the roles of different regions of the peptide on the rate of aggregation.
5. Find a signature of the initiation of peptide aggregation using artificial intelligence (AI) approaches on the AFM images.

References:

1. Bouter Y, Dietrich K, Wittnam JL, Rezaei-Ghaleh N, Pillot T, Papot-Couturier S, Lefebvre T, Sprenger F, Wirths O, Zweckstetter M, Bayer TA. N-truncated amyloid β (Aβ) 4-42 forms stable aggregates and induces acute and long-lasting behavioral deficits. Acta Neuropathol. 2013 Aug;126(2):189-205. doi: 10.1007/s00401-013-1129-2. Epub 2013 May 18. PMID: 23685882; PMCID: PMC3722453
2. Bouter Y, Dietrich K, Wittnam JL, Rezaei-Ghaleh N, Pillot T, Papot-Couturier S, Lefebvre T, Sprenger F, Wirths O, Zweckstetter M, Bayer TA. N-truncated amyloid β (Aβ) 4-42 forms stable aggregates and induces acute and long-lasting behavioral deficits. Acta Neuropathol. 2013 Aug;126(2):189-205. doi: 10.1007/s00401-013-1129-2. Epub 2013 May 18. PMID: 23685882; PMCID: PMC3722453.
3. Thomas, R. S.; Liddell, J. E.; Murphy, L. S.; Pache, D. M.; Kidd, E. J. An antibody to the β-secretase cleavage site on amyloid-β-protein precursor inhibits amyloid-β production. https://content.iospress.com/articles/journal-of-alzheimers-disease/jad00637.
4. **Nat. Struct. & Mol. Biol.** 17 (2010) 561–567

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