

## Exploring the Effects of Epigallocatechin-3-Gallate on the Disaggregation of Serum Amyloid A1 Fibrils

Natalie G. Horgan<sup>1</sup>, Anabela Djurovic-topalovic<sup>1</sup>, Hannah IR Charles<sup>1</sup>, Natsumi Kobayashi<sup>2</sup>, Tomoaki Murakami<sup>2</sup>, Jessica S. Fortin<sup>1</sup>

<sup>1</sup>Basic Medical Sciences, College of Veterinary Medicine, Purdue University, 625 Harrison Street, West Lafayette IN 47907

<sup>2</sup>Laboratory of Veterinary Toxicology, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu, Tokyo, Japan

**Introduction:** Serum Amyloid A1 (SAA1) is a major acute-phase protein involved in lipid metabolism, cytokine production, and immune responses. It is synthesized in the liver as a 122-amino acid protein, maturing into a 104-amino acid structure that forms a hexamer with a four-helix bundle. Importantly, SAA1 is a key precursor to amyloid A (AA), an insoluble protein that can build up in tissues. This accumulation leads to AA amyloidosis, a condition characterized by tissue damage and organ dysfunction resulting from chronic inflammation. Given these risks, our study explores potential strategies to disaggregate SAA1 fibrils isolated from domestic animals, with particular attention to the effects of epigallocatechin-3-gallate (EGCG), a green tea compound known for its anti-inflammatory and antioxidant properties.

**Methods:** The SAA1 fibrils were isolated from the cat spleen, chicken liver, and cow liver and incubated for 5 days in PBS (pH 7.4). To confirm the amyloidogenic properties of these fibrils, we conducted dynamic light scattering (DLS) and transmission electron microscopy (TEM) analyses of these fibrils (at ~0.3 mg/ml) with 0.25% DMSO and 200 to 400  $\mu\text{M}$  of EGCG.

**Results:** Our findings revealed that EGCG effectively disaggregates SAA1 amyloid fibrils isolated from domestic animals into non-toxic aggregates. Notably, EGCG caused a significant reduction in particle area in cow liver samples, decreasing from approximately 4.8  $\mu\text{m}^2$  to 0.5  $\mu\text{m}^2$ . A reduction was also observed in chicken liver samples, with particle area decreasing from approximately 1.8  $\mu\text{m}^2$  to 1.4  $\mu\text{m}^2$ . The soluble particle sizes of EGCG treated cow and chicken SAA1 fibrils were reduced from 1000 nm to 100 nm of diameter according to DLS. Significant ultrastructural morphological changes were observed with the cow SAA1 fibrils treated with EGCG.

**Conclusions:** Identifying compounds like EGCG that can disaggregate amyloid fibrils emphasizes their potential as therapeutic agents for treating inflammatory conditions and neurodegenerative diseases.