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Natural Product Research

Formerly Natural Product Letters

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/gnpl20

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To cite this article: Anna Lia Asti, Stefania Crespi, Teresa Rampino, Paola Zelini, Marilena Gregorini, Alessia Pascale, Nicoletta Marchesi, Stefania Saccucci, Carla Colombani, Sara Vitalini & Marcello Iriti (2023): Yet another *in vitro* evidence that natural compounds introduced by diet have anti-amyloidogenic activities and can counteract neurodegenerative disease depending on aging, Natural Product Research, DOI: 10.1080/14786419.2023.2192493

To link to this article: https://doi.org/10.1080/14786419.2023.2192493

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2023.2192493

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SHORT COMMUNICATION

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Yet another *in vitro* evidence that natural compounds introduced by diet have anti-amyloidogenic activities and can counteract neurodegenerative disease depending on aging

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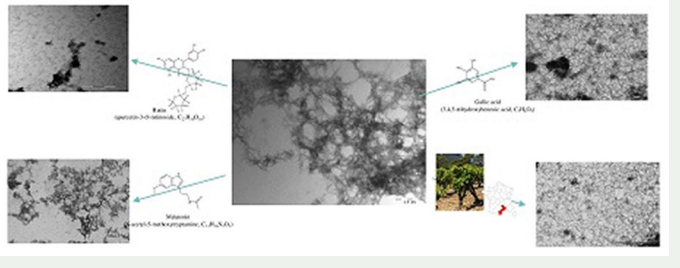
ABSTRACT

A major issue in Alzheimer's disease (AD) research is to find some new therapeutic drug which decrease Amyloid-beta (Aβ) aggregation. From a therapeutic point of view the major question is whether pharmacological inhibition of inflammation pathways will be able to safely reverse or slow the course of disease. Natural compounds are capable of binding to different targets implicated in AD and exert neuroprotective effects. Aim of this study was to evaluate the *in vitro* inhibition of $A\beta_{1\text{-}42}$ fibrillogenesis in presence of Gallic acid, Rutin, Melatonin and Provinols™ . We performed the analysis with Transmission and Scanning Electron Microscopy, and with X-ray microanalysis. Samples treated with Rutin, that arises from phenylalanine *via* the phenylpropanoid pathway, show the best effective result obtained because a significantly fibril inhibition activity is detectable compared to the other compounds. Melatonin shows a better inhibitory activity than Provinols™ and Gallic acid at the considered concentrations.



ARTICLE HISTORY
Received 5 December 2022
Accepted 11 March 2023

KEYWORDS
Amyloid-beta (Aβ); Alzheimer's disease (AD); polyphenolic compounds; Rutin; Gallic Acid; Melatonin; Provinols™

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/14786419.2023.2192493.

1. Introduction

Amyloid-β (Aβ) protein is the major component of senile plaques in Alzheimer's disease (AD) patients.

Aβ is an ancient conserved effector molecule of innate immunity, an antimicrobial peptide (AMP) (Soscia et al. 2010), The physiologically produced and circulating Aβ may have such functions because Aβ may initially be beneficial at the beginning of an infection, as an AMP helps to contain the original pathogen (Zaiou 2007).

As for all types of amyloid, aggregation, mature fibrils could be a neuroprotective measure to shift the balance away from soluble oligomers in an effort to reduce toxicity thereby reducing the number of exposed β-strands present which can induce a further aggregation.

Bacterial endotoxin may also promote the production or aggregation of Aβ (Asti and Gioglio 2014), Tau, and α-synuclein to give different neurodegenerative diseases.

From a therapeutic point of view the major question is whether pharmacological inhibition of inflammation pathways will be able to safely reverse or slow the course of disease.

Natural compounds are capable of binding to different targets implicated in AD and exert neuroprotective effects; aim of this study was to evaluate the inhibition of Aβ fibrillogenesis and aggregation with natural compounds. Gallic acid (3,4,5-trihydroxybenzoic acid, $C_7H_6O_5$) is a hydroxybenzoate arising from shikimc acid, the precursor of the aromatic amino acid pathway. This phenolic acid is widespread in many plants and has been investigated for its *in vitro* and *in vivo* antioxidant, anti-inflammatory and anticancer activities (Kahkeshani et al. 2019). Rutin (quercetin-3-*O*-rutinoside, $C_{27}H_{30}O_{16}$) is a flavonoid found in many food and medicinal plants. It arises from phenylalanine *via* the phenylpropanoid pathway and has been explored for a number of pharmacological effects, such as the antioxidant, antimicrobial, anti-inflammatory and anticancer activities, as well as vasoprotective, neuroprotective and cardioprotective effects (Ganeshpurkar and Saluja 2017; Iriti et al. 2017). Melatonin (N-acetyl-5-methoxytryptamine, $C_{13}H_{16}N_2O_2$) is a pleotropic molecule widespread among living organisms is an amphipathic molecule able to cross barriers as cell membranes and the blood–brain barrier, and, in addition, it possesses an intrinsic, powerful antioxidant capacity, scavenging the harmful reactive oxygen and nitrogen species (Varoni et al. 2016), Figure 1. Provinols™ is a commercial extract composed of polyphenols (95% of total polyphenols) of Cabernet-Sauvignon red wine collected in the Languedoc-Roussillon regions (South East of France) and selected for its antioxidant content (Varoni et al. 2013).

2. Results and discussion

2.1. TEM analysis of $Aβ_{1-42}$

$Aβ_{1-42}$ peptide was incubated for 72 hrs; in Figure S1 A a spontaneous *in vitro* fibrillogenesis of the peptide in long irregular, flexous, smooth paired fibrils; micellar particles are also detectable, Figure S1 B (arrow). In the early stage of AD pathogenesis Aβ may enter the mitochondria and induce ROS formation, oxidative stress and decreasing the levels of endogeneous antioxidants (Reddi 2006).

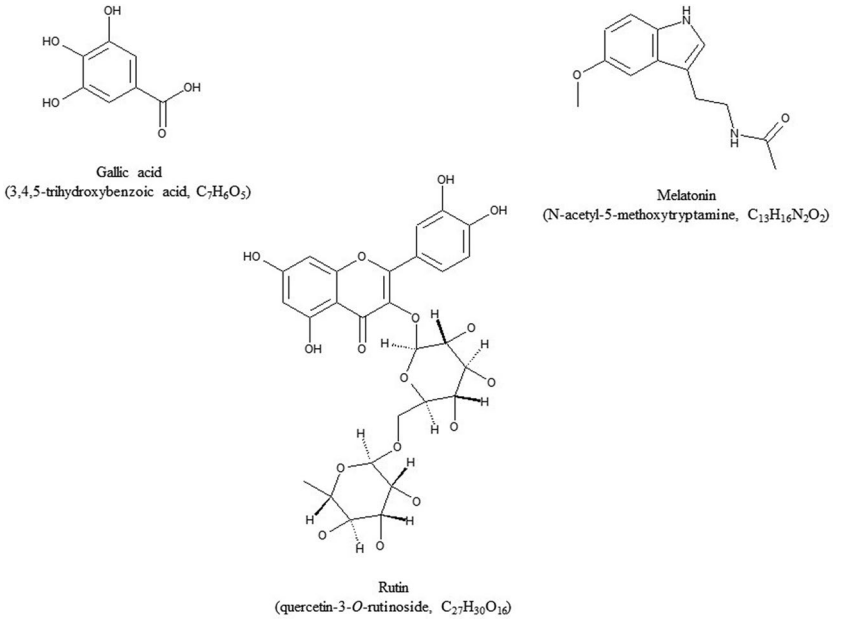

Figure 1. Natural compounds used in the antiamyloidogenic activity assay.

2.2. TEM analysis of $A\beta_{1-42}$ in presence of Gallic acid

$A\beta_{1-42}$ fibrils appear to be shorter in Figure S2 A and arranged in a network of fibrils with different nucleations centers. Molecular studies (Liu et al. 2013) have shown that Gallic acid interacts with Aβ aggregates and inhibits Aβ fibril formation by disrupting the Lys28-Ala42 salt bridge of Aβ. It seems that Gallic acid only gains its inhibitory potential after undergoing oxidation (Sakalauskas et al. 2020). The oxidized form is highly effective at inhibiting primary nuclei formation, while having no effect on fibril elongation.

2.3. TEM analysis of $A\beta_{1-42}$ in presence of Provinols™

Fibrils, Figure S3 A. are thinning, frials and less numerous than those observed in Figure S1 A. Red wine and its polyphenolic constituents possess lipid and lipoprotein-lowering effects (Agouni et al. 2009), there is a potential beneficial effects of the Provinols™ on beta-amyloid fibrils. Red wine drinking was not associated to a significantly decreased salivary antiradical activity, thus suggesting that the well-known antioxidant properties of polyphenols may be able to counteract, at least in part, the pro-oxidant effects of ethanol (Varoni et al. 2013).

2.4. TEM analysis of $A\beta_{1-42}$ in presence of Melatonin

Amorphous material, Figure S4 A, and not clearly identificable fibrils are detectable. Melatonin as an antioxidant, can attenuate Aβ-induced toxicity that is related to the oxidative stress (Wang et al. 2012). Melatonin modulates the regulatory network of

secretase expression thereby inhibiting amyloidogenic APP processing and Aβ production (Li et al. 2020). Biflavonoids inhibit Aβ fibrillogenesis and this results in the accumulation of non-toxic Aβ oligomeric structures.

2.5. TEM analysis of $A\beta_{1-42}$ in presence of Rutin

In Figure S5 A short fibrils and numerous oligomers are visible. Dietary flavonoid Rutin, can dose-dependently inhibit $A\beta_{42}$ fibrillization and attenuate the toxicity in SH-SY5Y cells (Wang et al. 2012). Especially nanocrystals are promising natural compounds to protect neurons from cell death and oxidative stress during PD. Polyphenol compounds has inhibitory effects on Aβ aggregation by binding hydrophobic β-sheet channels with their aromatic structure, and disturb Aβ hydrogen bond formation through the action of hydroxyls as electron donors (Convertino et al. 2009; Wang et al. 2012). In this study Rutin revealed a more effective result because it shows a significantly better fibril inhibition activity than the other tested compounds, while Melatonin seems to have a better inhibitory activity than Provinols™ and Gallic acid at the tested concentrations, Figure S6.

2.6. Scanning electron microscopy (SEM) analysis and backscattered electron (BSE)

Different 3D structures belonging to the different compounds analyzed, are visible in Figures S2 B, S3 B, S4 B, S5 B. SEM can provide information on surface topography and crystalline structure; BSE of all considered compounds gives modulated informations indicating the presence of different phases in the compound, Figures S2 C, S3 C, S4 C, S5 C.

2.7. EDS microanalysis of compounds

The elemental composition of each compounds is expressed in Figure S2 D, S3 D, S4 D, S5 D.

EDS can detect major and minor elements with concentration higher than 10 wt% and minor concentration between 1 and 10 (Makhlouf and Aliofkhazraei 2019). With EDS some elements are not detectable at all, such as hydrogen, helium and lithium. EDS was performed to detect elements with concentration higher than 10 wt% and minor concentration between 1 and 10. Some elements are not detectable at all, such as hydrogen, helium and lithium. For this reason hydrogen is not present.

3. Experimental section

See Supplemental material.

4. Conclusions

The compounds topic of this study might be useful in designing new drugs against fibril formation; small molecules to prevent the polymerization of Aβ could therefore be an effective therapeutic strategy for AD.

Being the average age of grown population, the use of natural compounds selected and introduced with the diet could counteract the incidence of neurodegenerative diseases linked to old age.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

Authors wish to thank Dr.Chiara Corpina and Dr.Melania Antonietta Sesta of Amyloidosis Research and Treatment Center Foundation, IRCCS Policlinico San Matteo Pavia, Italy Pavia.

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