Efficacy of Oleuropein Aglycone in the Treatment of Transthyretin-Amyloidosis

Abstract
Transthyretin (TTR) is an amyloidogenic protein implicated in diseases such as senile systemic amyloidosis (SSA) and familial amyloid polyneuropathy (FAP), both characterized by extracellular deposition of insoluble amyloid fibrils in heart, peripheral nerves and other organs. In particular, fibrils in FAP patients are composed of single-site mutant TTR and among the numerous pathogenic variants Leu55 → Pro55 (L55P) is highly amyloidogenic and forms amyloid fibrils in vitro. TTR fibrils have been considered directly responsible of tissue impairment in FAP and SSA, but the unstable fibril precursors are increasingly considered the main responsible of cell sufferance and tissue impairment in amyloid diseases. The recent introduction in the clinical practice of synthetic TTR-stabilizing molecules that reduce protein aggregation provides the rationale to search natural effective molecules able to interfere with TTR amyloid aggregation by hindering the appearance of toxic species or by favouring the growth of harmless aggregates. We focused our study on the ability of Oleuropein aglycone (OleA), the main phenolic component of the extra virgin olive oil, to inhibit the toxic effects of wt- and L55P- TTR amyloid. Our data offer the possibility to validate and optimize the use of OleA as itself or as a starting point to rationally design promising drugs that could enter in a clinical experimental phase.

Keywords: Transthyretin; Oleuropein aglycone; Amyloid; FAP; FAC

Abbreviations: TTR: Transthyretin; SSA: Senile Systemic Amyloidosis; FAP: Familial Amyloid Polyneuropathy; FAC: Familial Amyloid Cardiomyopathy; AD: Alzheimer’s Disease; APP: Amyloid Precursor Protein; hIAPP: Amylin or Human Islet Amyloid Polypeptide; wt-TTR: Wild-Type TTR; OleA: Oleuropein Aglycone; NDGA: Nordihydroguaiaretic Acid; EGCG: Epigallocatechin 3-Gallate; GM1: Monosialotetrahexosylganglioside

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Oleuropein Aglycone and Protein Aggregation
Diet in human health is no longer simple nutrition, but in light of recent epidemiological studies, it may have deep effects on modulating apoptosis, detoxification, appropriate gene response and protection against disease. Recently, nutrigenomics research suggested that it is not just deficiency of a particular element but also the presence in diet in adequate amount of various other compounds from fruits and vegetables that play a role in decreasing risk of a range of disease including cancer cardiovascular and neurodegenerative disease. Our laboratory focused attention on the main phenolic compound, Oleuropein aglycone (OleA), of extra-virgin olive oil. The pharmacological properties of olive oil, especially the olive fruit and its leaves have been recognized as important components of medicine and a healthy diet for their phenolic content [1]. OleA has several pharmacological properties such as antioxidant [1], anti-inflammatory [2] anti-atherogenic [3], anti-cancer [4], antimicrobial [5] and antiviral [6], and for these reasons, it is commercially available as food supplement. In addition, it has been shown to be cardioprotective against acute adriamycin cardiotoxicity [7] and has been shown to exhibit anti-ischemic and hypolipidemic activities [8]. Another important aspect to be considered is that the OleA interaction with cell
membranes would increase its local concentration. This aspect is particularly relevant when considering the beneficial effects that would be attained above critical concentration of the molecule.

Recently, most of the research on OleA was focused on its anti-amyloidogenic activity, instead it resulted be able: to interfere in vitro with amyloid fibril formation of two proteins, Amylin, or human islet amyloid polypeptide (hIAPP), and Aβ42, associated with type 2 diabetes and Alzheimer’s disease (AD), respectively [9,10]; studies in vivo, using C. elegans as a simplified invertebrate model of AD [11], showed that this polyphenol skips the appearance of toxic oligomers promoting peptide aggregation into aggregates devoid of cytotoxicity [12]. Finally, we recently showed that a dietary supplementation of OleA strongly improved the cognitive performance of the TgCRND8 mouse model of AD; mice showed remarkably reduced plaque deposits and microglia migration to the plaques for phagocytosis [13].

Data obtained with cultured cells confirmed that OleA is also able to induce autophagy, possibly by acting on the mTOR pathway [13]. OleA was more effective than Oleuropein and hydroxytyrosol (they are also effective but to a lesser extent) as inhibitor of Tau fibrillization [14]. OleA was also shown to modify Amyloid Precursor Protein- (APP) processing, increasing the formation of the non-amyloidogenic and neuroprotective sAPPα fragment and to decrease Aβ oligomers in HEK695 cell supernatants by increasing matrix metalloproteinase-9 (MMP-9) secretion [15]. These results are very promising and pave the way to a more extensive investigation on OleA ability to inhibit toxic amyloid aggregation of many other amyloid-associated peptides/proteins. Indeed, the consensus idea that the protein misfolding is the cause of several different diseases and the high similarity of amyloid aggregates originated from different protein/peptides, gives the opportunity to develop common therapies for these devastating diseases. The present review will describe the biological effects of OleA with particular attention on the molecular mechanism underlying the protective action against TTR amyloidosis toxicity.

Transthyretin Related Amyloidosis

TTR is one of the 25 known proteins that are capable of forming amyloid fibrils in vivo. TTR amyloidosis is a systemic disorder characterized by the extracellular deposition of amyloid fibrils composed of TTR. TTR amyloidosis includes senile systemic amyloidosis (SSA), familial amyloid cardiomyopathy (FAC), familial amyloid polyneuropathy (FAP), and central nervous system selective amyloidosis (CNSA). Over 80 disease-causing mutations in the TTR gene are known in FAP, most of which reduce tetramer stability increasing the fraction of aggregation-prone unstable dimeric and monomeric protein. However, although specific mutations are required for FAP development, different genetic factors may govern the age of onset and the occurrence of disease anticipation [16]. Instead, fibrils in FAP patients are composed of single-site mutant TTR and among the numerous pathogenic variants Leu55→Pro55 (L55P) is highly amyloidogenic and forms amyloid fibrils in vitro. Treatment of familial TTR amyloidosis has historically relied on liver transplantation as a crude form of gene therapy [17]. As TTR is primarily produced in the liver, replacement of the liver gene carrying the mutation with the normal form is able to reduce the mutant TTR levels in the body to < 5% of pre-transplant levels. Some mutations, however, cause CNS amyloidosis, and due to their production by the choroid plexus, these forms do not respond to liver transplantation.

Without adequate therapy, TTR amyloidosis results in a fatal outcome and lead to death within 10-15 years mainly following heart complications. Indeed, the presence of TTR aggregates in the heart parenchyma, may represent a basis for the occurrence of rhythm disturbances and conduction alterations often seen in patients with TTR amyloidosis. However, besides liver and heart transplantation, there is no effective medical treatment to enhance or to block disease progression.

On this point, it also appears of growing importance to search for compounds able to interfere with protein aggregation either by stabilizing native states against unfolding and by skipping the growth of toxic aggregation intermediates providing tools to counteract, or to slow down, the appearance and progression of diseases.

Transthyretin and Polyphenols

Several natural polyphenols have been reported to act on different amyloidogenic proteins inhibiting amyloid formation. In particular, for TTR, the attention is focused on some natural polyphenols (Figure 1) such as curcumin, nordihydroguaiaretic acid (NDGA) and epigallocatechin 3-gallate (EGCG) that have structure similarities with T4. These studies showed that all these compounds stabilize the TTR and inhibit amyloid aggregation by three different means: EGCG strongly suppresses the dissociation of the TTR tetramer maintaining it as a soluble protein; curcumin induces the oligomerization of TTR in a characteristic homogeneous population of non-toxic “off-pathway” small aggregates and NDGA moderately reduces the amount of aggregated TTR [18]. Furthermore, the oligomers and the intermediate species formed with the treatment of EGCG and curcumin are not toxic to the neuronal cells [19].
Oleuropein Aglycone Reduces Transthyretin Fibrils Formation

The knowledge of the mechanism by which polyphenolic compounds block amyloid formation is of considerable importance, as amyloid aggregation is a common feature of many degenerative diseases including Alzheimer’s, type II diabetes, Parkinson’s, Creutzfeld-Jacob’s, Huntington’s and many others. The selection and development of small molecules able to inhibit the formation of toxic protein assemblies or to reduce their ability to bind to cell membrane could have a considerable clinical application, making a determination of the mechanism by which polyphenols disrupt aggregated conformations essential for structure-activity assays. Recent papers suggested that aromatic interactions favour molecular recognition of amyloidogenic sequences by enhancing the directionality and orientation needed for the ordered self-assembly process and hence fibril assembly kinetics [22]. On the other hand, it has been reported that several polyphenolic compounds are able to interact with amyloidogenic aromatic residues hindering β-system stacking [23,24] and inhibiting the elongation phase of fibril growth or the assembly of large oligomers without interfering with early nucleation events [25]. Our biophysical analysis [26] showed that OleA is not able to inhibit TTR aggregation of both wt- and L55P-TTR but it reduces the exposition of protein aromatic residues during the amyloidogenic process. We found that the presence of OleA during wt-TTR and L55P-TTR aggregation pathway induced an increase of Trp quenching, suggesting that the effect of OleA in stabilizing meta-stable intermediate states relies on the reduction of the overall surface hydrophobicity of the aggregates (Figure 2). OleA does not prevent TTR aggregation by itself but, rather, it is effective in counteracting mature fibrils growth, a behaviour comparable with previously data shown with tau [27], amylin [9] and Abeta peptides [10]. The reported data suggest that OleA induces some remodelling of the supramolecular structure of the growing aggregates. This implies a mechanism of action different from that merely ascribable to some stabilization of the protein native structure hindering misfolding and aggregation, as observed for other anti-amyloidogenic compounds [28,29].

Oleuropein Aglycone is Cytoprotective against TTR Toxicity

Considering that amyloid aggregates can bind to a large number of biological molecules that range from glycosaminoglycans (e.g. GM1) and nucleic acids to a variety of proteins and lipids, the change in membrane dynamics observed for GM1, following the binding of amyloid aggregates, may imply that amyloids can potentially harm all those cellular mechanisms that base their efficiency on molecules mobility [30]. Actually, growing evidence shows that amyloid aggregates can alter membrane mobility of a number of proteins and other plasma membrane components, leading either to a gain or to a loss of function. For example, the binding of Sup35 amyloid fibrils to the plasma membrane can cause an accumulation of Fas receptors associated with GM1 with subsequent activation of the extrinsic apoptotic pathway [31]. On the other hand, sequestration of neurotransmitter receptors, such as the metabotropic glutamate mGlur5, by Ab1-42 oligomers has been shown to impair intracellular calcium levels and synaptic network activity [32]. In this context, an alteration of GM1 mobility may compromise its regulatory role in neurodevelopment and neuroprotection [33,34], or influence cellular pathways linked to raft dynamics.

It is widely reported that a main mechanism of amyloid aggregate cytotoxicity requires the primary interaction with the cell membrane, which results in functional and/or structural perturbation of the latter. Emerging evidence points to fibrils/membrane interaction as a key factor of the cytotoxic potential of amyloid aggregates [35,36]. Indeed, alterations of membrane fluidity resulting from TTR-aggregates interaction with neuronal cells were previously reported to be a key event in protein cytotoxicity [37]; this evidence results also from our study where both wt- and L55P-TTR fibrils appeared able to interact specifically with membrane GM1 of cardiomyocyte cells line [26]. Our study indicates that OleA protection results from a different mechanism whereby its interaction with TTR does not stabilize the tetramer structure but hinder TTR transition to an amyloid structure, rather, OleA interferes with TTR fibrillation by stabilizing oligomer-like intermediates and changing the surface properties of TTR/OleA aggregates. Accordingly, the presence of OleA during TTR aggregation induces a significant reduction of TTR aggregates-GM1 interaction, with consequently loss of cytotoxicity (Figure 3).

These data suggest that OleA, or its molecular scaffold can be a good starting point to design novel therapeutic strategies for
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