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Secondary Metabolites of Lichens as Both Anti-aggregative and Antioxidant Agents in Tauopathies

Alberto Cornejo[1*] **and Carlos Areche**[2]

Escuela de Tecnología Médica, Facultad de Medicina, Universidad Andrés Bello, Sazie 2315, 8370092-Santiago-Chile

Departamento de Química, Facultad de Ciencias, Universidad de Chile, Ñuñoa, 8320000-Santiago-Chile

[*]**Corresponding author:** Alberto Cornejo, Faculty of Medicine, School of Medical Technology, Andrés Bello University, Sazié 2315, First Floor, Santiago 8370092, Chile, Tel: +56227703610; E-mail: alberto.cornejo@unab.cl

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Abstract

This commentary described our findings of parietin, an anthraquinone, isolated from *Ramalina terebrata* as inhibitor of tau protein. Moreover, we considered important to link tauopathies with reactive oxygen species since oligomers and fibril-forming elements are responsible for activating reactive oxygen species, which cause inflammatory response and neurodegeneration. Together, we considered important to find naturally occurring compounds that might be able to stop aggregation and reduce ROS cells damage.

Keywords: Tauopathies; Secondary metabolites; Lichens; Reactive oxygen species; Inflammatory response; Neurodegeneration

Tauopathies and Neurotoxicity

Taoupathies are neurodegenerative disorders involving tau protein, such as progressive nuclear palsy (PSP), corticobasal degeneration (CBD), Pick´s disease among others. Tau is an unfolded protein which is found mainly in axons of mature and physiologically is involved in microtubule stability and axonal transport [1]. However, once tau becomes hyperphosphorylated it detaches from microtubule starting the aggregation process [2,3]. Pathological tau aggregates are able to activate glia cells that release cytotoxic factors, which cause pro inflammatory cytokines such as TNF-α, IL-1 and IL-6 and chemokines [4]. Tau phosphorylation is increased in both physiologically and pathological state [5], however phosphorylation is increased during development, suggesting that process is needed for neuronal plasticity [6]. Tau phosphorylation is an event highly regulated by kinases and phosphatases. Its balance is deregulated due to several stimuli such as oxidative stress [7]. Tau is able to form paired helical filaments closely related to neurofibrillary tangles [8,9].

Hyperphosphorylated tau protein is found in patients suffering with tauopathies in cerebrospinal fluid (CNS), which correlates well with hypocampal atrophy [10]. In addition, tauopathies have tau hyperphosphorylated as a hallmark; however, the degree of phosphorylation differs among them. In addition, it is important to notice that there is no single phosphorylation site associated to a particular tauopathy [5]. Moreover, tau has the propensity to form aggregates, since inside the full length protein (441 aminoacids) resides a region known as 4R (four microtubule binding domain) containing two hexapeptides ^{275}VQIINK^{280} and ^{306}VQIVYK^{311} both associated to β sheet formation [11]. Increased tau phosphorylation decreased its binding for microtubules. These species prone to form aggregates which are toxic in both cell and transgenic mouse model [12,13]. Despite that fact, there are evidences showing that soluble species and pre fibrils which are more related to toxicity [14]. An interesting UV raman spectroscopy study showed that at early stages, within the first hour, fibrillar aggregates possess a mixture of β-sheet and disordered content. Afterward, the UVRR spectra shows a consolidation in fibril structure, augmenting the content of β sheet [15], interesting is to remark that toxicity apparently relies on β sheet content [16].

Tauopathies and Reactive Oxygen Species

Reactive oxygen species (ROS) are reactive molecules such as hydroxyl (OH·), superoxide (O2·) and nitric monoxide (NO·). Besides, other molecules like hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$) are not free radicals; however they are reported to generate free radicals [17,18].

In general, cells exposed to reactive oxygen species can overproduce free radicals that promotes oxidative damage on macromolecules, which can lead to pathological disorders such as stroke, chronic inflammation, also this species contribute to develop neurodegenerative disorders such as tauopathies. [18,19]. Once cells are in contact with toxic species, they are able to synthetize several enzymes in order to clear them, however residual superoxides and peroxides remain [18]. Moreover, there are evidences showing that ROS were first described in Pick´s disease and CBD [20]. It was also described that ROS associated to tauopathies, such as malondialdehyde (MDA) or 4-hydroxynonenal (HNE), a specie linked to polyunsaturated fatty acids. [21,22].

In contrast, antioxidants can prevent ROS that are able to induce injury. However, there are synthetic antioxidants that have shown either to induced cell toxicity or mutagenesis [23]. Thus it is timely to search for naturally occurring antioxidants. Hence, several antioxidant properties have been described associated to flavonoids, hydroxycinnamic derivatives, catechins, curcumin among others [24,25].

Importantly, the phenolic ring is the main moiety involved in scavenging ROS, metal chelator and modulates both the endogenous enzymatic-non enzymatic antioxidant system involved in neurodegenerative disorders including tauopathies [26].

Lichens as antioxidants and tau inhibitors source

Lichens are symbiotic association between a mycobiont (fungus) and either algae or cyanobacterium [27-29]. Besides lichens species are able to produce important secondary metabolites [30]. A remarkable feature of lichen species is their antioxidant capacity that resides in their phenolic moieties [31]. Interestingly, some depsides and depsidones isolated from lichens have shown antioxidant capacity [32-34]. Besides this, neuroprotective effect and cytotoxic potential have been described in *Cetraria islandica* and *Vulpicida canadensis* [35]. Two Xanthones isolated from *Pyrenula japonica* have a potent antioxidant capacity as compared with α-tocopherol and 2,6-di(tert-butyl)-4-methylphenol (BHT) [36]. Moreover, ramalin isolated from Antartic *Ramalina terebrata* presented scavenging activity and it inhibits tyrosine enzyme activity. In addition, ramalina possess a very little toxicity in keratinocyte and fibroblast [37,38].

A β-orcinol depsidone, stictic acid and salazinic acid showed neuroprotective effect on U373MG cell line by diminishing ROS production. These compounds would be useful as antioxidant agents in Alzheimer´s disease [39], but it is important to notice that none of them have been tested as anti-tau inhibitors. Alternatively, a derivative plant polyphenol, curcumin, has been useful, since curcumin exerts a pleiotropic effect by combining both anti-aggregate and antioxidant activities.

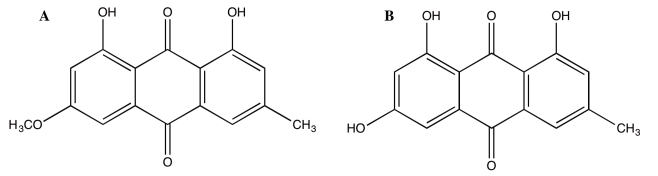

Figure 1: Anthraquinones compound tau inhibitors. **A)** Parietin IC_{50} 72 μM over tau four microtubule binding domain (4R). **B)** Emodin IC_{50} 0.3 μM over tau K-18 construct.

Recently, we have characterized that parietin, an anthraquinone, isolated from *Ramalina terebrata*, lichen collected in the Antarctic region of "Península Fildes", has effect on tau aggregation [40]. Docking studies of parietin and ^{306}VQIVYK^{311} hexapeptide suggest that parietin bind steric zippers preventing β-sheet assembly [40]. According to the docking model, there are both types of interaction: hydrogen bond (HB) among phenolic groups, methoxy motif and lysine side chains. Besides this, hydrophobic interaction also occurs between methyl group of methoxy substituent and valine [40]. Interestingly, another anthraquinone, emodin, which inhibits tau aggregation has lower IC_{50} instead of parietin, we hypothesize that could be due to methoxy group at C-3 position in parietin (Figure 1). Moreover, another anthraquinone derived from plant, rhein, can reverse DNA methylation and de-suppression of Klotho, which has an essential role in anti-renal fibrosis in a mouse model [41,42]. Furthermore, in a senescence-accelerated mouse model, rhein reduces levels of Aβ [43], however both rhein and emodin are poorly active against Aβ *in vitro* aggregation assays [44]. Although, another anthraquinone, emodin, exerts profound effect over tau aggregation [45], and its scavenging capacity is lower as compared with alaternin [46], but there is no evidence that alaternin inhibits tau aggregation. Considering that oligomers or fibril-forming motif, exert their influence over inflammatory system, it would be interesting to find molecules isolated from natural sources that combine both anti-aggregative and scavenging properties, thus it would be a proper way to address drug design. In addition, it is interesting to mention that not only tauopathies exerts their damage on aggregation, since in neurodegenerative disorders such as Parkinson´s disease, α-synuclein is also prone to form aggregates and fibrils, and their toxicity is also linked to β sheet formation.

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