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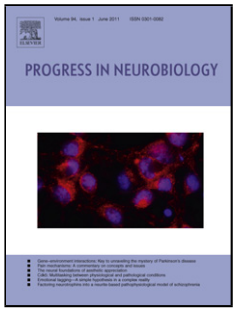

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Exploiting the therapeutic potential of ready-to-use drugs: repurposing antibiotics against amyloid aggregation in neurodegenerative diseases

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Abstract

Neurodegenerative diseases are chronic and progressive disorders that affect specific regions of the brain, causing gradual disability and suffering that results in a complete inability of patients to perform daily functions. Amyloid aggregation of specific proteins is the most common biological event that is responsible for neuronal death and neurodegeneration in various neurodegenerative diseases. Therapeutic agents capable of interfering with the abnormal aggregation are required, but traditional drug discovery has fallen short. The exploration of new uses for approved drugs provides a useful alternative to fill the gap between the increasing incidence of neurodegenerative diseases and the long-term assessment of classical drug discovery technologies. Drug re-profiling is currently the quickest possible transition from bench to bedside. In this way, experimental evidence shows that some antibiotic compounds exert neuroprotective action through anti-aggregating activity on disease-associated proteins. The finding that many antibiotics can cross the blood-brain barrier and have been used for several decades without serious toxic effects makes them excellent candidates for therapeutic switching towards neurological disorders. The present review is, to our knowledge, the first extensive evaluation and analysis of the anti-amyloidogenic effect of different antibiotics on well-known disease-associated proteins. In addition, we propose a common structural signature derived from the antiaggregant antibiotic molecules that could be relevant to rational drug discovery.

Abbreviations

Aβ, amyloid β peptide

AD, Alzheimer′s Disease

ALS, amyotrophic lateral sclerosis

Amb, Amphotericin B

BBB, blood-brain barrier

CJD, Creutzfeld Jakob disease

CSF, cerebral spinal fluid

DCS, D-Cycloserine

DOX, Doxorubicin

GA, Geldanamycin

GAPDH, glyceraldehyde-3-phosphate dehydrogenase

GS, Gramicidin S

HD, Huntington's diseases

LB, Lewis bodies

LPS, lipopolysaccharide

MCS, minimal common structure

MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

NFT, Neurofibrillary tangles

PD, Parkinson′s Disease

PrP, Prion protein

SNpc, substantia nigra pars compacta

SOD1, superoxide dismutase

ThT, thioflavin T

TSEs transmissible spongiform encephalopathies

1.1 The structural fingerprint of neurodegeneration

Neurodegenerative diseases such as Alzheimer's (AD), Parkinson's (PD), prion, amyotrophic lateral sclerosis (ALS) and Huntington's diseases (HD), among others, are currently being classified under a similar molecular and cellular mechanism whereby protein aggregation appears to be involved in triggering and spreading neuronal death in specific brain regions (Soto et al., 2003; Takalo et al., 2013). In fact, twenty-six years ago, the aggregation of a peptide called β-amyloid (or Aβ) became the dominant model to explain the molecular basis of AD. This model was coined the "amyloid cascade", and this concept was used to explain the deposition of different proteins associated with more than 60 disorders (Beyreuther and Masters, 1991; Hardy and Higgins, 1992; Selkoe, 1991). Over the years, this hypothesis has been modified to implicate soluble prefibrillar intermediates as the neurotoxic species. Nevertheless, the process of protein amyloid aggregation remains the main feature of neurodegenerative diseases until today (Beyreuther and Masters, 1991; Hardy and Allsop, 1991; Hardy and Higgins, 1992; Karran et al., 2011; Sami et al., 2017; Selkoe, 1991 Selkoe et al., 2016).

Amyloid aggregates include a structural superfamily of highly organized supramolecular structures that share a unique array of sheets; strands are stacked perpendicularly to the fibril growth axis and compose a novel quaternary structure referred to as cross-β. This amyloid specific structure was found in postmortem biopsies from patients with most neurodegenerative diseases and thus became the histopathological hallmark of these pathologies (Kayed et al., 2003). The cross-β arrangement is irrespective of the nature of the precursor proteins, and the formation of amyloid structures is not an exceptional phenomenon associated with a small number of polypeptides, but it reveals a well-defined structural state of any protein when its

metastable native status is perturbed (Chiti and Dobson, 2006; Dobson, 2003; Eisenberg and Jucker, 2012). Moreover, this fibrillar architecture can be adopted not only by globular cytosolic proteins but also by membrane proteins (Stroobants et al., 2017), which reinforces the theory that amyloid aggregates are a generic state of proteins.

The similarity between cross-β arrangements is highlighted by the finding that *ex vivo* and synthetic fibrils made of different proteins have similar high-resolution X-ray diffraction patterns even when proteins are heterologously expressed (Sunde et al., 1997a; Sunde et al., 1997b). Therefore, exceptional *in vitro* models for the study of the amyloid aggregation process are available.

Of note, a similarly kind of aggregation-associated disease resulting from the formation of intermolecular linkages is the familial encephalopathy with neuroserpin inclusion bodies (FENIB). Serpins, a superfamily of protease inhibitors, present an elegant and unique mechanism of action based in profound conformational transitions and translocations (Belorgey et al 2007; Lomas et al 2002; Takehara et al 2010). Unfortunately, their structural flexibility makes them highly susceptible to mutation and prone to aggregate by forming abnormal intermolecular linkages (Belorgey et al 2007; Lomas et al 2002; Takehara et al 2010). The progressive accumulation within the endoplasmic reticulum of these aggregates cause cell dysfunction resulting in several diseases that share a common mechanism of polymerization, actually known as serpinopathies (Belorgey et al 2007; Lomas et al 2002).

Wild type serpins have a metastable conformation made-up by 3 β‑sheets (A to C), 9 α‑helices and an exposed mobile reactive centre loop (RCL) which carries a pseudosubstrate involved in the docking of the target proteinase. However, point mutations can destabilize β-sheet A allowing the insertion of the RCL of another serpin molecule. Thereby, polymers result from

sequential reactive-loop insertion through an intermediate with an destabilized β-sheet (Belorgey et al., 2007; Lomas et al., 2002; Takehara et al., 2010).

In the FENIB pathology the progressive accumulation of polymers of mutant neuroserpin in the form of inclusion or Collin's bodies in the cerebral cortex leads to cognitive deficits and presenile dementia (Belorgey et al., 2007; Bradshaw et al., 2001; Lomas et al., 2002). Likewise, although the mechanism and the kinetic of formation differ between amyloids and neuroserin-polymers their associated dementias share conformational features being protein aggregation the injurious consequence of the formation of intermolecular linkages (Lomas et al., 2002).

1.2 Molecular pathway of neurodegeneration

The amyloid aggregation pathway is a sequential multistep reaction in which a protein in its soluble native state undergoes a self-association process that ends in a fibrillar state after moving through different soluble and insoluble intermediates (Fig 1). It is characterized by an initial lag phase that reflects a nucleation process, a growth or elongation phase, and a steady state (Wood et al., 1999) (Fig 1). The intermediate species of the amyloid reaction constitute a dynamic and heterogeneous population of particles with different sizes, structures, morphologies and biophysical and functional properties. Moreover, depending on the context, the amyloid reaction may follow alternative pathways that lead to the formation of either toxic (on-pathway) or non-toxic (off-pathway) species (Ehrnhoefer et al., 2008) (Fig 1).

From a kinetic point of view, the rate-limiting step of this process is the nucleus formation. The nuclei can grow from their ends and can behave as "seeds" to hasten the aggregation of other native monomers (Fig. 1). The seeds are thought to be produced during the lag phase (Soto et al.,

2006), while the recruitment of monomeric proteins around the nucleus is produced in the second step or elongation phase.

The nuclei, as well as higher aggregates such as protofibrils, can diminish the length of the lag phase when added to a native protein solution (Fig. 1B). This seeding property has a crucial impact on the degenerative process in cell cultures, tissues, and the brain (Brundin et al., 2008).

Figure 1

1.3 Identity of the toxic species. Shifting between the fibril and its precursors

The presence of amyloid deposits, the most obvious pathognomonic characteristic of neurodegenerative disease, supported for many years the idea that amyloid fibrils are the primary inducers of pathogenesis. However, another hypothesis has recently emerged from evidence that demonstrates that the extent of fibrillar amyloid plaque deposition does not correlate with Alzheimer's disease pathogenesis (Terry, 1996). Moreover, a significant number of non-demented individuals have similar amounts of amyloid plaques as diseased patients (Terry, 1996). These findings appeared to relegate the amyloid theory. Nonetheless, *in vitro* evidence suggests that pre-fibrillar species, rather than mature amyloid fibrils, are likely to be the primary pathogenic agents in neurodegenerative diseases; this evidence reinforced the relevance of the amyloid theory in neurodegenerative processes (Haass and Selkoe. 2007). In this sense, the structural characteristics, which resemble the typical membrane pore-forming protein arrangements on pathway intermediates of the aggregation process, have gained attention due to their capacity for disrupting membranes and inducing mitochondrial dysfunction or oxidative damage (Avila et al., 2014; Bennet et al., 2005; Conway et al., 2000; Cremades et al., 2012; Danzer et al., 2007; Huang et al., 2015; Takahashi et al., 2008; Winner et al., 2011). Notably,

some mature insoluble amyloid aggregates may not be fully devoid of toxicity, since it has been recently reported that they are responsible for triggering inflammation in PD (Gustot et al., 2015). Moreover, methods commonly used to separate oligomeric species are not efficient enough, and a significant number of short fibrils may remain with oligomeric species; therefore, the nature of the primary toxic species is still not well known. Furthermore, Melki (2017) proposes that the toxicity between fibrils and oligomeric species be compared considering the identical number of particles and not polypeptide concentration. In this comparison, fibrillar assemblies were found to be more toxic than oligomers (Pieri L. et al., 2012; 2016). Moreover, fibrils might also be able to recruit the soluble forms of analogue proteins *in vitro* and *in vivo* (El-Agnaf et al., 1998; Hansen et al., 2011; Luk et al., 2009; Melki 2015; Nekooki-Machida et al., 2009; Novitskaya et al., 2006; Sanders et al., 2014; Yang W. et al., 2002).

In addition, growing evidence shows that oligomeric species may be transferred from cell to cell (Emmanouilidou et al., 2010; Jang et al 2010; Lee et al., 2005). Furthermore, in recipient cells, transferred species behave as seeds and trigger the formation of small aggregates made from intracellular native protein (Chai et al., 2013; Desplats et al., 2009; Volpicelli-Daley et al., 2011).

1.4 Biological usefulness of aggregation: Pushing the system to alternative ways

Amyloid fibril formation from soluble proteins is not an exclusive event of neurodegenerative conditions, since nature has exploited the extraordinary physical properties of cross-β arrangements in many contexts. The physiological forms of amyloids, known as "functional amyloids", play protective and adaptive roles across the phylogenetic tree (Bian et al., 2000; Chapman et al., 2002; Claessen et al., 2003; Hammer et al., 2008; Kenney et al., 2002; Wickner, 1994).

In humans, amyloid fibrils composed of the pigment cell-specific premelanosome protein (PMEL) are extensively spread in skin, hair and eyes, and form the scaffold for melanin in melanocytes (Watt et al., 2013). Human functional amyloids were also described in the factor XII activation process, which specifically impacts on the kallikrein-kinin system that regulates inflammation, blood pressure, and pain without inducing coagulation (Maas et al., 2008). Additionally, a specific sugar from the extracellular matrix triggers glyceraldehyde-3-phosphate dehydrogenase (GAPDH) amyloid aggregation, and some intermediate species from this reaction kidnap *in vitro* toxic species related to neuronal death, which suggests that these aggregates may exert a protective role *in vivo* (Avila et al., 2014).

Both sides of amyloid aggregation, from essential roles of functional amyloids to their implication in neurodegeneration and systemic amyloidosis, suggest that the protein aggregation process must be strictly regulated in order to avoid deleterious effects (Hammer et al., 2008).

A naturally occurring mechanism for the regulation of amyloid toxicity via structural tuning was elegantly illustrated in the biology of Microcin E492 (Mcc), an amyloid-forming antimicrobial peptide (Shahnawaz and Soto, 2012). Depending on the environmental condition, this peptide can be found in two interconvertible forms: i) toxic active soluble oligomers capable of altering the membrane integrity of susceptible bacteria, and ii) non-toxic insoluble amyloid fibrils. During growth, Mcc-producing bacteria synthesize soluble toxic oligomers that target competing microorganisms, ultimately causing their demise. Once the competitors have been eliminated, pore-forming Mcc oligomers reversibly assemble into non-toxic amyloid fibrils that remain a reservoir of the toxic species (Shahnawaz and Soto, 2012).

One unanswered question in this model refers to the specific conditions or cofactors that "track the road" in the protein aggregation pathway toward the production of either protective or harmful amyloid species.

1.5 Neurodegenerative diseases in the 21st century. Novel strategies for therapy

In the brain specifically, the protheostatic mechanisms that regulate amyloid aggregation pathways are probably very tightly regulated since any imbalance might involve the onset of a neurodegenerative process. Although factors known to alter this balance are well studied, the complete landscape of this process remains unknown.

On the other hand, the increase in longevity in the human population situates neurodegenerative diseases as a critical challenge to health care systems throughout developed countries. In fact, according to a systematic study only on Alzheimer's disease, 7.7 million people are affected in the US alone, and this number is estimated to rise to 13.5 million by 2050. (http://www.alz.org/documents_custom/trajectory.pdf). Additionally, the actual expenditure of dementia care in the UK almost matches the combined cost of cancer, heart disease and stroke (http://www.alz.co.uk/research/files/WorldAlzheimerReport.pdf). Regarding PD, the situation is analogous to AD, since approximately 4.6 million Western European patients were diagnosed in 2005, and that number is expected to increase to approximately 9 million in 2030 (Dorsey et al., 2007). These data highlight the urgent need for the identification of effective neuroprotective therapies in order to avoid the collapse of the healthcare system. Therefore, treatments must directly target the underlying disease pathogeneses as the primary method of altering the inexorably progressive clinical course of these diseases. Likewise, the inhibition of abnormal protein aggregation should be the main target of therapies for amyloid-associated diseases.

Unfortunately, despite the significant investment in research and drug development, to date, all attempts have failed.

Although promising compounds have demonstrated an ability to stop or revert protein aggregation *in vitro* or in animal models, unfortunately, most of them fail in preclinical or clinical trials because preclinical assays cannot always account for all physiological differences between animal models and humans (Pardridge et al., 2005). Moreover, most drugs currently described as neuroprotective in clinical trial registration databases that reach phase three are mainly aimed at neurotransmitter release regulation or metabolism instead of the inhibition of the abnormal protein aggregation process (https://clinicaltrials.gov/). Therefore, available therapeutic approaches are more palliative than curative.

In this context and from a neurological point of view, any compound that is capable of interfering with the amyloid aggregation process, either by preventing the production or promoting the clearance of the pathogenic species, would be a valuable candidate for the development of an effective therapy against neurodegenerative diseases. In this sense, an encouraging alternative strategy could be drug repurposing. This method encompasses the use of a drug that has already passed a significant number of toxicity and safety tests. Therefore, the time required to reach clinical trial status is expected to be dramatically decreased. Likewise, the identification of an old, well-known medicine with novel antiaggregating activity on proteins associated with neurodegenerative disorders would be groundbreaking.

Since pioneer studies showed that leprosy patients who received chronic treatment with antibiotics displayed significantly decreased prevalence of dementia (Chui et al., 1994), great expectations regarding the role of antibiotics as neuroprotective agents have arisen. Aside from their anti-bacterial activity, compelling experimental evidence currently shows that many other

features of antibiotics, including anti-inflammatory, anti-aggregating, and antioxidant properties, can be beneficial for the treatment of neurological disorders (Forloni et al., 2009; Gonzalez Lizarraga et al., 2017; Noble et al., 2009; Santa-Cecilia et al., 2016; Stoilova et al., 2013; Sultan et al., 2013).

In the present review, evidence regarding the impact of antibiotics on the pathological aggregation of disease-associated proteins is extensively examined. In addition, due to the chronicity of neurodegenerative diseases, we discuss the risk of adverse events during long-term exposure to different antibiotics and analyze the feasibility of repurposing them for human clinical therapy.

2.1 Alzheimer's Disease: a tale of two proteins finally connected in one road

AD, the major cause of age-related dementia worldwide, is currently considered a proteopathy or protein misfolding disease since two brain proteins are involved in neuronal death. Neurofibrillary tangles composed of amyloid aggregates of hyperphosphorylated microtubule-associated protein tau (MAPT) are currently considered pathological hallmarks of AD. Moreover, since the concentration of tau in the cerebral spinal fluid (CSF) and the neurofibrillary tangle (NTF) load in the brain strongly correlate with the clinical progression of the disease, they have been proposed as biomarkers for AD (Tapiola et al., 1997). Extracellular deposits of amyloid β peptides (Aβ) known as senile plaques have also been implicated in the pathogenesis of AD. The most accepted amyloid hypothesis of AD links the aggregation pathway of both proteins and suggests that Aβ is the primary cause of dementia due to its ability to induce NFT formation, which occurs downstream of Aβ aggregation (Hardy et al., 2002; Ittner et al., 2010; Kosik, 2006). Nevertheless, growing evidence indicates that oligomeric species, which precede

the appearance of fibrillar aggregates, are more involved in neuronal loss than NFT or senile plaques themselves (Iqbal et al., 2010; Maeda et al., 2006; Sepulveda-Diaz et al., 2015; Takashima, 2013).

Clinically, AD is characterized by a progressive deterioration of memory and cognitive functions that finally leads to complete incapacity and death (Querfurth et al., 2010; Zhao et al., 2013). Despite tremendous advances in our knowledge of the disease, its accurate causes and pathogenesis are not entirely understood, and unfortunately, there are no effective therapies for halting or slowing the neuronal damage associated with the disease. Thus, the need for the development of novel therapeutic agents capable of preventing or halting the progression of the amyloid cascade of tau, Aβ peptide, or both, is crucial. Below, we will discuss the neuroprotective properties of antibiotics (Chessell et al., 1991; Forloni et al., 2001; Namba et al., 1992) in an attempt to elucidate their ability to interfere with the proteopathic process of AD.

2.1.1 Tetracyclines

The interest in the anti-amyloidogenic activity of tetracyclines arises from the observation that these widely prescribed, blood-brain barrier (BBB)-crossing and well-tolerated antibiotics (Klein et al., 1995) could abolish the toxic effects of Aβ in a transgenic *Caenorhabditis elegans* model of AD (Diomede et al., 2010). In this model, the expression of the Aβ peptide leads to the formation of β-sheet enriched structures, the intracellular accumulation of which induces a paralysis phenotype (Diomede et al., 2010). However, feeding transgenic *Caenorhabditis elegans* with either tetracycline, doxycycline or minocycline triggered a disassembling of the β-sheet enriched structures of Aβ and significantly decreased the accumulation of the oligomeric species (Diomede et al., 2010). This finding is in agreement with previous observations from Forloni and co-workers (2001) indicating that tetracyclines, such as doxycycline and

minocycline, can inhibit the aggregation pathway of Aβ1-42 and disassemble mature amyloid fibrils of the peptide. Furthermore, these antibiotics strongly increased the susceptibility of Aβ1-42 amyloid fibrils to trypsin digestion (Forloni et al., 2001; Sirangelo et al., 2010), which suggests a different supramolecular arrangement in the presence of the antibiotic. In this way, Airoldi and colleagues (2011) demonstrated that the presence of tetracycline induced the formation of novel disordered and non-homogeneous aggregates of Aβ1-42 with significantly less toxicity in N2a cell lines. These tetracycline-induced aggregates belong to off-pathway species since they cannot achieve the final product of the amyloid aggregation reaction, *i.e.,* mature fibrils (Airoldi et al., 2011) (Fig. 4). Furthermore, tetracycline interacts with early oligomers of Aβ1-42, changes their supramolecular organization and pushes the aggregation reaction to the amorphous way, generating large non-fibrillar aggregates that lack toxicity (Airoldi et al., 2011) (Fig. 4).

Regarding doxycycline, Costa and colleagues (2011) demonstrated that in the presence of this antibiotic, Aβ peptide follows the same aggregation pathway and leads to the formation of non-toxic, cross beta lacking, amorphous aggregates that remain in a soluble state. These authors also demonstrated that doxycycline has no effect on the neurotoxicity of pre-aggregated β-sheet enriched oligomers in a cell model of AD. This indicates that although the antibiotic could be useful for the prevention of the formation of toxic oligomers of Aβ, once those species are assembled, it is incapable of counteracting their deleterious effects (Costa et al., 2011).

Concerning the role of tau protein in AD, Noble and colleagues (2009) found that minocycline can reduce the formation of tau aggregates in primary cortical neurons and enhance cell survival. In this respect, minocycline treatment diminished caspase-3 activation and the level of aggregation-prone caspase-3-cleaved tau fragments (Noble et al., 2009). Moreover, when treated

with minocycline, the tangle-forming transgenic mice displayed significantly diminished levels of phosphorylated tau and tau aggregates (Noble et al., 2009).

Notably, concerning the potential use of tetracyclines in neurology, a clinical trial that encompassed more than one hundred patients with probable AD and mild to moderate dementia showed that co-treatment with daily doses of doxycycline and rifampicin (also known as rifampin) for three months significantly diminished cognitive decline and dysfunctional behavior (Loeb et al., 2004). However, these encouraging results were later challenged by a multicenter, blinded, randomized, factorial controlled trial, the DARAD trial, which indicated that neither doxycycline nor rifampicin were effective in significantly slowing the progression of degeneration in AD patients over a twelve-month treatment period (Molloy et al., 2013). Nevertheless, these trials were performed with patients diagnosed with AD; therefore, amyloid oligomeric species were probably already formed before antibiotic treatment.

2.1.2 Gramicidin S

In addition to tetracyclines, a few other antibiotics have been shown to possess neuroprotective properties. In this regard, Luo and co-workers (2013) demonstrated that Gramicidin S (GS), a cyclic, wide-spectrum, peptide antibiotic with potent antimicrobial activity against Gram-positive and Gram-negative bacteria and some fungi (Kondejewski et al., 1996; Luo et al., 2013), can block the aggregation pathway of Aβ *in vitro*. Furthermore, it can disassemble pre-formed amyloid fibrils. By using *in silico* docking studies, Luo and colleagues (2013) proposed a novel interaction between the cyclic decapeptide structure of the antibiotic and the Aβ peptide; in this model, GS assumes a β-sheet conformation that interacts with those from the Aβ oligomers, enables the binding of the antibiotic to the growing Aβ-protofilaments, and blocks amyloid fibril formation (Fig. 2).

Figure 2.

Unfortunately, Gramicidin S is highly toxic to red blood cells. It causes hemolysis in humans; therefore, its applications have been restricted to topical uses (Finch et al., 2010). For this reason, Gramicidin S-derivatives that display reduced hemolytic activity but conserving antimicrobial action as well as antiaggregating action have been developed (Kapoerchan et al., 2010; Luo et al., 2013). Despite the finding that Gramicidin S and its analogues are not ready-to-use drugs, they provide new strategies for the development of inhibitors against Aβ fibril formation. The finding that some derivatives are still capable of inhibiting the aggregation pathway of Aβ1-40 indicates that the structural determinants within the molecule of GS responsible for the anti-aggregating properties are different from those that cause the hemolytic activity of the antibiotic (Luo et al., 2013). It is important to notice that the ability of Gramicidin S and gramicidin-like compounds to disassemble preformed amyloid fibrils should be tested in terms of toxicity since the disruption of fibrils could produce new oligomeric species. Therefore, more toxicological assays regarding the safety of these derivatives are required before their application in humans.

2.1.3 D-Cycloserine

D-Cycloserine (DCS) is an antibiotic widely prescribed for the treatment of multidrug-resistant *Mycobacterium tuberculosis* infections and has been shown to exert beneficial effects in patients with neurological disorders. Moreover, compelling evidence indicates that DCS exerts neuroprotective activity and may act as a cognitive enhancer in AD (Billard et al., 2007; Chessell et al., 1991; Schneider et al., 2000). Additionally, Chaturvedi and co-workers (2015) showed that when the Aβ-42 peptide was subjected to aggregating conditions in the presence of DCS, the antibiotic significantly attenuated the toxicity of the peptide and increased cell viability.

Molecular docking studies showed that DCS interacts with residues Phe19, Phe20 and Asp23 of the Aβ-42 peptide, leading to the formation of DCS/Aβ-42 complexes with low cross-β-sheet conformation content and stabilized by hydrophobic interactions and hydrogen bonding. These complexes block the progression of the amyloid pathway and thus prevent the formation of toxic Aβ species (Chaturvedi et al., 2015).

2.1.4 Amphotericin B

Amphotericin B (AmB) is an antifungal antibiotic that kills yeast by binding to the essential lipid ergosterol, leading to the formation of ion channels with concomitant membrane permeabilization (Gray et al., 2012). AmB is one of the few compounds known to delay the progression of prion disease in animals (Hartsel et al., 2003), and thus, its activity as an antiamyloidogenic compound was also tested with Aβ peptides. By using Congo Red as a specific dye for the detection of amyloid structures, Hartsel and colleagues (2003) demonstrated that AmB can bind to the aggregation-prone region Gly25-Met35 from Aβ-42 and inhibit its fibrillization. However, Smith and co-workers (2009) reported that although AmB effectively binds to β-sheet enriched soluble oligomers of Aβ-42, it does not have any effect on the aggregation kinetics of fibril formation. These controversial results may be explained by taking into account that a potential inhibitor molecule and the amyloid specific dye used may compete for the same binding site within the growing aggregates, and thus, the binding of one of them may interfere with the binding of the other (Smith et al., 2009).

Consequently, a lower signal from the amyloid specific dye does not necessarily imply an inhibition of the fibrillization process, but it could indicate the binding of a compound to the amyloid aggregates and the displacement of the dye from them, although the cross-β structure of the particles may not be affected. Therefore, to appropriately characterize the anti-aggregating

property of any compound, several complementary techniques, including circular dichroism, infrared spectroscopy, dynamic light scattering and thioflavin T (ThT) and Congo red spectroscopies, should be performed (Smith et al., 2009). These considerations clearly show that more experimental data regarding the effects of Amphotericin B on Aβ-42 are required in order to clarify its potential as an anti-aggregation agent against Aβ misfolding. In any case, since AmB exerts dose-dependent nephrotoxicity (Goldman et al., 2007; Soler et al. 2008), harmless derivatives are required to avoid injurious secondary effects on patient health during chronic treatment. Notably, as we will subsequently discuss, Soler and colleagues (2008) developed AmB derivatives that lack toxicity and antimicrobial activity but retain the anti-aggregating action on Prion protein (PrP). If this anti-aggregation activity is extensible to Aβ, then these AmB derivatives could be suitable molecules for the treatment of AD. However, at least to our knowledge, no study on this subject has been performed. Although AmB is not currently a ready-to-use drug, knowledge derived from the AmB:Aβ peptide interaction could prove essential for intelligent drug design.

2.1.5 Anthracycline

Anthracycline antibiotics were discovered in the late 1930's and were found to exert potent activity against Gram-positive microorganisms (Rabbani et al., 2005). Nonetheless, the detection of their antitumor activity in 1963 redirected their application as valuable molecules in cancer therapy (Booser et al., 1994). Later, in the 1990's, the observation that a derivative of the anthracycline antibiotic doxorubicin (DOX) induced amyloid resorption led Merlini and colleagues (1995) to study the effects of this molecule on amyloidosis. In this respect, it was found that it binds tightly to several natural amyloid fibrils, including those purified from the AD brain, mainly through hydrophobic interactions (Merlini et al., 1995; Tagliavini et al., 1997).

Moreover, the binding of the DOX derivative to the growing amyloid fibril decelerates the fibrillization process, and it was hypothesized that the antibiotic could enhance the clearance of amyloid structures by increasing their solubility (Merlini et al., 1995). However, no experimental data to support this hypothesis have been reported. On the other hand, taking into account compelling evidence that indicates that the most toxic species are the soluble β-sheet-enriched oligomers, the disassembly of amyloid fibrils may not be, from the clinical point of view, a valuable alternative unless the soluble species that were formed lacked toxicity. In addition, clinical evidence demonstrated that doxorubicin as a chemotherapeutic agent entails an increased risk for cardiomyopathy due to cumulative dose-dependent cardiotoxicity (Volkova et al., 2011). Overall, the cumulative dose of doxorubicin, its intrinsic toxicity and its associated risk for developing heart failure has restricted its clinical application in human health and, at least to our knowledge, the development of DOX derivatives that lack toxicity has not yet been achieved.

2.1.6 Rifampicin

Rifampicin is a BBB-crossing semi-synthetic macrocyclic antibiotic, and it is probably the most studied antibiotic regarding neurodegenerative disorders. It exerts strong antiaggregating activity, which significantly decreases the accumulation and toxicity of intracellular Aβ oligomers in cultured cells (Tomiyama et al., 1997; Yulug et al., 2014). Moreover, in cell-free conditions, rifampicin inhibits the toxic oligomer formation of Aβ, tau, and α-synuclein, which suggests that the formation of noxious species from these proteins involves a similar mechanism and a common structural determinant (Umeda et al., 2016). This inhibition of the oligomerization process is also supported by the observation that a rifampicin-treated group of leprosy patients showed a decreased incidence of AD and a reduction in amyloid plaque formation (Chui et al., 1994; Namba et al., 1992). However, Umeda and colleagues (2016) observed no significant

decrease in amyloid deposition in aged Tg2576 mice in the presence of rifampicin, which suggests that the antibiotic does not affect preformed fibrils in amyloid plaques. Nonetheless, rifampicin did significantly diminish the accumulation and toxicity of intracellular Aβ oligomers in cell models and in *in vitro* assays, improve cognitive function, and decrease the accumulation of Aβ and tau oligomers in mouse models of AD (Umeda et al., 2016).

From a structural point of view, several mechanisms were proposed to explain the neuroprotective effect of rifampicin. The primary effect is the inhibition of protein oligomerization. In this sense, Tomiyama and co-workers (1994) showed that rifampicin inhibits the aggregation process of Aβ1-40 *in vitro* and prevents the formation of toxic species. Subsequently, it was found that rifampicin also abolished Aβ-related neurotoxicity in a cell model of AD (Tomiyama et al., 1996). By binding to oligomers, rifampicin would avoid a potential oligomer–membrane interaction and prevent membrane disruption by pore formation, which is considered one of the potential toxic mechanisms of these species (Demuro et al., 2005; Quist et al., 2005). The oligomer-induced membrane impairment allows Ca^{2+} influx into the cells, causing mitochondrial dysfunction and reactive oxygen species (ROS) generation (Mancuso et al., 2006; de Moura et al., 2010). On the other hand, since it was demonstrated that the oxidation of Aβ by free radicals induces peptide fibrillization (Dyrks et al., 1992), antioxidant agents may exert anti-aggregating effects on the Aβ peptide. Alike, the radical-scavenging activity of rifampicin has been shown to play key roles in the inhibition of the amyloid cascade, which leads to neurotoxicity, and in induced neuronal death in cell models of AD (Tomiyama et al., 1996; Tomiyama et al., 1997). Therefore, on one hand, the radical-scavenging function of rifampicin prevents Aβ oxidation and diminishes its aggregation rate; on the other hand, rifampicin protects cells against Aβ-induced intracellular ROS production and

increases neuronal survival (Tomiyama et al., 1994; Tomiyama et al., 1996; Tomiyama et al., 1997).

The evidence described above, together with the ability of this antibiotic to suppress microglial activation in different models, strongly indicates that rifampicin is a promising, quite ready-to-use drug (Bi et al., 2011; Umeda et al., 2016). However, the main side effect of this antibiotic, the hepatotoxicity (No authors, 2008), might limit its long-term use. Moreover, rifampicin leads to a decrease in the efficiency of drugs that are concomitantly prescribed with it. Thus, considering that elderly people, who are usually polymedicated, are the main population affected by neurodegenerative disorders, this side effect must be taken into account. An alternative that could be explored is an alteration in the administration from oral to intranasal to improve the safety and efficiency of this neuroprotective drug. Likewise, models for the intranasal administration of drugs to deliver neuroprotective molecules straight to the brain have recently been described (Prediger et al., 2011)

2.1.7 Anisomycin

Anisomycin (*a.k.a.* flagecidin) is a potent protein synthesis inhibitor that can induce apoptosis. Wang and colleagues (2008) found that anisomycin exerts strong effects on tau, promotes the conversion from native to pathological forms, and consequently increases the level of aggregation-prone pathogenic hyperphosphorylated tau in mouse neuroblastoma cells (N2a). Moreover, MAPK and GSK-3 kinases were also significantly activated by anisomycin (Wang et al., 2008). GSK-3 is a well-known kinase involved in the phosphorylation of tau (Liu et al., 2002). In this respect, the increase in the tau phosphorylation level was fully abolished by lithium chloride, a specific inhibitor of GSK-3, without affecting MAPK activity, which indicates that anisomycin induced the hyperphosphorylation of tau through the activation of the

GSK-3 kinase (Wang et al., 2008). Interestingly, the effects of anisomycin on memory and behavior are closely related with those distinctive for AD and dementia, *i.e.,* anisomycin impaired social-recognition memory when administered in the olfactory bulb and dorsal hippocampus in Swiss adult mice (Penna et al., 2014). Furthermore, anisomycin can also induce amnesia and impair short-term and long-term memory formation when injected in the hippocampus (Qi et al., 2009; Remaud et al., 2014). However, these notable effects of anisomycin have been attributed to its inhibitory activity on protein synthesis, which is required for the consolidation of reactivated memory (Remaud et al., 2014; Sharma et al., 2012), rather than to its action on tau. However, more overwhelming evidence to support this hypothesis is required; *i.e.,* it would be valuable to test whether a derivative compound that lacks anti-translational activity exerts the same effects on memory and behavior as anisomycin. Interestingly, the anisomycin-induced apoptotic cascade was found to take place before a significant reduction in protein synthesis was observed (Iordanov et al., 1997; Rudy et al., 2006). Notably, an anisomycin concentration that reduces the protein synthesis rate only by 10% can enhance the activation of the stress-activated pro-apoptotic protein kinases (SAPKs) and cJun NH2-terminal kinases (JNKs) at 50% of their maximum in cell culture, which indicates that anisomycin may exert other effects on cells that are independent of its translational inhibitory action (Iordanov et al., 1997; Rudy et al., 2006). As previously suggested by Rudy and colleagues (2006), if these apoptotic effects take place in neurons that support memory, then the anisomycin-induced amnesia could not be exclusively attributed to its inhibitory action on protein synthesis. Be that as it may, more experimental evidence is required to clarify the role and action of anisomycin on memory and behavior.

2.2 Synucleinopathies: A tale of a single protein involved in multiple disorders

Synucleinopathies are progressive neurodegenerative disorders with no current treatment, including pathologies with an overwhelming clinical prognosis such as PD, dementia with Lewy bodies, and multiple system atrophy (Spillantini et al., 1997; Spillantini et al., 1998; Tong et al., 2009). Notably, the common event among these neurodegenerative disorders is the abnormal accumulation of α-synuclein in the form of amyloid aggregates in neural or glial cells (Duda et al., 2000).

PD is the synucleinopathy disorder with the highest prevalence and represents a major challenge to health care systems (Reglodi et al., 2017). Histopathologically, PD is characterized by the loss of dopaminergic neurons in the midbrain region known as the *substantia nigra* pars compacta (SNpc) and by the presence of cytoplasmic inclusions in surviving neurons called Lewis bodies (LB). These inclusions are predominantly formed by α-synuclein in a misfolded fibrillary stage (Bennett et al., 2005; Nussbaum et al., 2003; Ruzza et al., 2014; Spillantini et al., 1998). Since this abnormal aggregation has been suggested to be one of the earliest and key steps during the development of the disease (Avila et al., 2014; Bennett et al., 2005; Ruzza et al., 2014), there is a continuous search for compounds that exert an anti-aggregating action on α-synuclein. Currently, a growing body of evidence suggests that the neurodegenerative process observed in PD is not only caused by protein aggregation but also by mitochondrial dysfunction and neuroinflammation. Nonetheless, α-synuclein aggregation appears to be the primary event that triggers all other injurious processes. Furthermore, neuroinflammatory processes and mitochondrial impairment can promote protein aggregation. As a result, the deleterious effects of neuronal toxins such as oligomeric species, ROS, and proinflammatory factors are continuously amplified and generate an endless, noxious circle (Bennett et al., 2005; Dutta et al., 2008; Gustot

et al., 2015; Jiang et al., 2013; Nakamura et al., 2013; Onyango et al., 2008; Plotegher et al., 2014; Pukass et al., 2014; Ruzza et al., 2014; Zhang et al., 2000). In this context, antibiotics and their surprising neuroprotective features, including anti-inflammatory, anti-aggregating and antioxidant properties (Gonzalez-Lizarraga et al., 2017; Santa-Cecilia et al., 2016), have gained the attention of the scientific community. Moreover, accumulating evidence suggests that antibiotics may constitute the starting point for the development of an efficient treatment for PD (Egeberg et al., 2016; Ruzza et al., 2014). We have currently identified five antibiotics that exert antiamyloidogenic activity against α-synuclein that are described and discussed below.

2.2.1 Geldanamycin

Geldanamycin (GA), a benzoquinone ansamycin antibiotic, has been shown to abolish neurotoxicity in a fly model of PD mainly by decreasing the aggregation of α-synuclein (Auluck et al., 2002). Furthermore, it has been demonstrated that GA selectively affects molecular chaperones, inhibits Hsp90 and up-regulates Hsp70 (Sittler et al., 2001). Interestingly, compelling evidence indicates that Hsp70 modulates protein misfolding and aggregation and confers protection against the degenerative processes observed in many neurological diseases, including HD, PD and spinocerebellar ataxias (Auluck et al., 2002; Chan et al., 2000; McLean et al., 2004; Sittler et al., 2001). In this respect, McLean and co-workers (2004) found that the up-regulation of Hsp70 by GA significantly decreased the misfolding of α-synuclein and diminished its aggregation rate in human H4 neuroglioma cells. Nevertheless, the treatment of diseased cells with GA has no effect on pre-formed α-synuclein inclusions, which indicates that the antibiotic blocks the initial steps of the amyloid pathway but cannot disassemble mature fibrils (McLean et al., 2004). Interestingly, similar effects of GA on other amyloidogenic disease-causing proteins were reported. *i.e,.* Sittler and colleagues (2001) found that GA treatment induces a heat shock

response activation that leads to an increased expression of Hsp70 and consequently to the inhibition of the self-assembly of the huntingtin exon 1 protein. This finding is in agreement with previous data from Chan and co-workers (2000) that indicate that the overexpression of Hsp70 and Hsp40 counteracts polyglutamine-induced toxicity in a *Drosophila melanogaster* model system for Machado-Joseph disease (MJD/SCA3). Taken together, experimental data supports a protective role of GA in neurodegenerative disorders, including PD and HD, by preventing the formation of toxic oligomers and thus enhancing neuron survival (McLean et al., 2004).

Despite the finding that GA diminishes α-synuclein aggregation through heat shock proteins and could be administered in combination therapies with other molecules that target the cross-β structure of amyloid aggregates, its toxicity hinders its clinical development. However, structural analogues such as 17N-allylamino-17-demethoxygeldanamycin (17-AAG, KOS-953, or tanespimycin), 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG, or alvespimycin), IPI-493, and retaspimycin (IPI-504) are now being evaluated in clinical trials, and these results are forthcoming.

2.2.2 Ceftriaxone

Ruzza and co-workers (2014) found that ceftriaxone, a BBB-crossing, widely prescribed, well tolerated β-lactam antibiotic, exhibits surprising neuroprotective properties in an *in vitro* model of PD, mainly through the inhibition of the aggregation pathway of α-synuclein. Moreover, computational docking studies indicate that ceftriaxone binds with high affinity to monomeric α-synuclein, and its binding site is located at the C-terminal region of the protein (Ruzza et al., 2014). Furthermore, the ceftriaxone/α-synuclein interaction leads to an increased compactness of the monomeric form of the protein that negatively affects its kinetics of aggregation, thus blocking the formation of toxic amyloid aggregates. Notably, Ruzza and co-workers (2014) also

found that ceftriaxone might diminish the 6-OHDA-induced up-regulation of α-synuclein expression. In this regard, working together, the protective effects of ceftriaxone at different levels enhance neuron survival in the 6-OHDA cell model of PD (Ruzza et al., 2014).

From a functional point of view, α-synuclein is not only a pathological protein but may also exert a physiological role. Likewise, it was recently reported that monomeric α-synuclein interacts with brain ATP synthase and increases its efficiency, which suggests that in non-pathological conditions this protein would be a regulator of mitochondrial bioenergetics (Ludtmann et al., 2016). However, considering the putative function of native monomeric α-synuclein, the binding of the antibiotic to the physiological form of the protein suggests that ceftriaxone may interfere with its role in cellular metabolism. The application of ceftriaxone in therapy must be carefully examined despite its capacity to increase neuronal survival in PD models.

2.2.3 Rifampicin

Interestingly, in addition to its protective properties against Aβ-induced toxicity, rifampicin has also been shown to counteract key pathological features of PD. In this sense, Li and co-workers (2004) found that rifampicin exerts a potent anti-aggregating action on native α-synuclein *in vitro* by stabilizing the monomeric form of the protein. Moreover, rifampicin is also capable of disassembling the pre-formed fibrils of α-synuclein to yield a heterogeneous mixture of soluble oligomers and monomers (Li et al., 2004). Nonetheless, the toxicity of the species formed from the rifampicin-induced disassembly of mature fibrils was not analyzed in the study by Li and co-workers (2004). Additionally, Xu and colleagues (2007) demonstrated that rifampicin blocks the deleterious effects of the neurotoxin 1-Methyl-4-phenyl pyridinium (MPP+) in a PC12 cell model of PD. MPP+ treatment induces the formation of aggregated species of α-synuclein within neurons and leads to decreased viability. Nevertheless, these multimeric species of α-synuclein

were not observed in the presence of rifampicin. Moreover, neuron survival was significantly enhanced by rifampicin, which suggests that its neuroprotective properties are probably associated with its anti-aggregating action on α-synuclein (Xu et al., 2007). This is in agreement with a previous study by Kilic and colleagues (2004) that showed that rifampicin treatment increased the survival of dopaminergic neurons after MPP+ intoxication in a cell model of PD. On the other hand, Jing and colleagues (2014) found that the expression of glucose-regulated protein 78 (GRP78), an essential constituent of the cellular defense system devoted to the removal of misfolded proteins, is up-regulated by rifampicin. Interestingly, compelling evidence indicates that GRP78 prevents apoptosis in neurons (Goldenberg-Cohen et al., 2012; Jiang et al., 2012; Reglodi et al., 2017). Therefore, the effect of rifampicin on GRP78 expression and consequently on the accumulation of misfolded α-synuclein may explain, at least in part, the anti-apoptotic protective properties of the antibiotic (Jing et al., 2014; Reglodi et al., 2017). However, as already mentioned in the preceding section (2.1.6), the toxicity of rifampicin must be carefully evaluated prior to the selection of this antibiotic for chronic therapy.

2.2.4 Tetracyclines

Regarding tetracyclines, a recent report from a 15-year Danish Nationwide Cohort Study (Egeberg et al., 2016) found an increased incidence of PD in patients with ocular rosacea. However, tetracycline therapy for the treatment of rosacea significantly reduced the risk of PD (Egeberg et al., 2016). In addition, the results from our group show that doxycycline interferes with the pathologic cycle involved in synucleinopathies at the aggregation level by binding to early multimeric α-synuclein species and inducing their reshaping into non-toxic off-pathway oligomers that do not evolve into fibrils (Gonzalez-Lizarraga et al., 2017). This reshaping mechanism diminishes the hydrophobic surface of the oligomeric species and alters their ability

to destabilize biological membranes, cell viability, and seeding capacity (Gonzalez-Lizarraga et al., 2017). A non-trivial observation was made regarding the dose of doxycycline necessary to interfere with the pathological aggregation of α-synuclein. According to our studies, an equimolar concentration of doxycycline to α-synuclein would be suitable to confer a protective effect. Therefore, considering that the α-synuclein concentration in cerebrospinal fluid is approximately 0.12 nM and that doxycycline at subantibiotic doses (20–40 mg/day) reaches the brain at a concentration of approximately 3 μM, the antibiotic level would be high enough to confer neuroprotection (Gonzalez-Lizarraga et al., 2017). In addition, previous and complementary studies from our group demonstrated that this antibiotic could diminish the impairments produced by the intrastriatal administration of 6-OHDA by inhibiting microglial and astrocyte expression in a 6-OHDA mouse model of PD (Lazzarini et al., 2013). Furthermore, we also demonstrated the efficiency of doxycycline in the modulation of the neuroinflammatory response in lipopolysaccharide (LPS)-activated primary microglial cells in culture, as a model of neuroinflammation (Santa Cecilia et al., 2016). It is expected that this anti-inflammatory effect acts synergistically with the antiamyloidogenic action of doxycycline on α-synuclein pathogenesis, which led us to propose its repurposing as a ready-to-use neuroprotector, and a multifunctional molecule against synucleinopathies, specially PD (Gonzalez-Lizarraga et al., 2017).

Despite the fact that more experimental data are required, compelling evidence suggests that tetracyclines, especially doxycycline, may be valuable alternatives as therapeutic agents to prevent the formation of toxic aggregates in synucleinopathies.

2.2.5 Rapamycin

A few studies showed that rapamycin, a BBB-crossing antibiotic that is widely used in human health to avoid rejection during organ transplantation due to its immunosuppressive activity, confers protection and enhances neuron survival in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD (Liu et al., 2013). Interestingly, these protective properties of rapamycin depend, at least partially, on its capability to decrease α-synuclein aggregation and to promote oligomers clearance trough increased autophagy (Liu et al., 2013; Reglodi et al., 2017).

The adverse effects of this antibiotic in long-term treatments are well known since it is commonly used in the medical clinic as a modulator of the immune system in transplants. Rapamycin decreases glucose tolerance and induces insensitivity to insulin (Lamming et al., 2012), and it could increase the risk of type 2 diabetes (Johnston et al., 2008). Moreover, lung toxicity is a well-reported complication associated with rapamycin therapy (Chhajed et al., 2006; Filippone et al., 2011). On the other hand, according to FDA prescribing information, due to its immunosuppressive activity, rapamycin increases the susceptibility for the development of skin cancers and lymphoma (https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/021083s058,021110s075lbl.pdf).

Considering the number of adverse effects, a balance between the efficacy and toxicity of rapamycin led us not to consider it as a ready-to-use drug for chronic treatment against neurodegenerative disorders. However, the rapamycin molecule is an encouraging starting point for the development of safer structural analogues with neuroprotective properties.

2.3 PrP-Related Diseases

Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are a group of progressive and lethal neurodegenerative disorders pathologically linked to the Prion protein (PrP a.k.a. PrP^{C}), and include Creutzfeld Jakob disease (CJD), Gerstmann-Sträussler-Sheinken (GSS) diseases, Kuru, and fatal insomnia (FI) (Collins et al., 2004; Corato et al., 2009; Forloni et al., 2009). Pathologically, these diseases are characterized by massive neuronal death and by the formation of vacuoles that cause a "sponge-like" appearance in the brain (De Luigi et al., 2008; Soler et al., 2008). Within these pathologies, the most common human disorder is CJD, which represents 90% of PrP-related diseases (De Luigi et al., 2008). Despite the finding that the causes include sporadic, infectious, or genetic origins and may vary between these fatal disorders, in all of them, the conversion from native PrP^{C} into the pathogenic PrP^{Sc} form plays a central role during the development of the pathologies. In this respect, the misfolding and subsequent aggregation of the protease resistant PrP^{Sc} form leads to the formation of toxic oligomers, which dramatically affects cellular function and induces extensive neuronal death (De Luigi et al., 2008). Moreover, it was found that diverse prion strains with distinct biological properties can propagate themselves within the same host, leading to the formation of strain-specific PrP^{Sc} oligomers that conserve the biological features of each particular strain (Bruce et al., 2003; Cronier et al., 2007; Safar et al., 1998). Unfortunately, despite extensive research and the tremendous advances achieved in this field, an effective therapeutic agent has not yet been developed. Nonetheless, the neuroprotective properties of antibiotics provide hope for the development of therapies to fight these disabling and fatal disorders.

2.3.1 Amphotericin B

Pioneer studies from Xi and co-workers (1992) showed that the antifungal polyene antibiotic AmB diminished the accumulation of pathological PrP^{Sc} in the brains of scrapie-infected hamsters and delayed the appearance of clinical signs of the disease, which increased the survival time after infection. Moreover, it was found that the neuroprotective properties of AmB depend on its capability to inhibit the conversion of the cellular PrP^{C} precursor to the pathologic form, PrP^{Sc} (Cronier et al., 2007; Mangé et al., 2000a; Mangé et al., 2000b; Xi et al., 1992). In addition, PrP molecules have been shown to be attached through a glycosyl phosphatidylinositol (GPI) molecule to the plasma membrane and are concentrated in diverse detergent-resistant microdomains (DRM), where the conversion of PrP^{C} into PrP^{Sc} is thought to take place (Gorodinsky et al., 1995; Mangé et al., 2000b). Interestingly, it was found that AmB can also modify the properties of the DRM to prevent the transition from PrP^{C} to the pathological form of the protein (Mangé et al., 2000b). In addition, through structure-function studies of AmB derivatives, the key determinants within the antibiotic molecule required for its diverse effects were identified. In this respect, the polyene structure of the antibiotic appears to be crucial for cellular toxicity and for the antiprion and antifungal activities (Soler et al., 2008). Moreover, the double bond C28-29 in the AmB molecule has been shown to be essential for the antiprion action since its removal drastically decreased the antiprion activity of the antibiotic (Soler et al., 2008). Conversely, AmB derivatives that lack exocyclic carboxyl groups can inhibit the conversion from PrP^{C} into PrP^{Sc} in cell culture, which indicates that those groups within the antibiotic molecule are dispensable for the antiprion effect. Interestingly, Soler and colleagues (2008) were able to isolate an AmB derivative, 16-descarboxyl-16-methyl-19-O-(6-deoxyhexosyl)-19-O-desmycosaminyl-amphotericin (16-19B), which displays reduced toxicity in cell culture and lacks antifungal activity but exerts increased antiprion action. These observations suggest that

those properties are not intrinsically related and highlight the potential of antiprion AmB-based drugs in the development of an effective therapy for PrP-related diseases.

2.3.2 Tetracyclines

Concerning tetracyclines, Forloni and co-workers (2013) demonstrated that, in addition to its anti-aggregating action on Aβ and α-synuclein, doxycycline can also inhibit the conversion of PrP^{C} to the pathological form PrP^{Sc}. Moreover, the pretreatment of homogenates from prion-infected brains with doxycycline diminished their infectivity, delayed the development of the pathology in the recipient animal after intracranial inoculation and increased the survival time (Forloni et al., 2013; Forloni et al., 2009). Furthermore, it was reported that tetracycline, doxycycline, and minocycline significantly prolonged the survival of hamsters infected with the 263K scrapie strain either intracerebrally, subcutaneously or intramuscularly, and these antibiotics still exert protective properties even if they are administered after the infection when the first symptoms appear (De Luigi et al., 2008; Forloni et al., 2009).

Regarding the mechanism of action of tetracyclines on TSEs, Tagliavini and colleagues (2000) found that tetracyclines can bind to synthetic PrP peptides and Prp^{Sc} *in vitro*, inhibit the self-assembly of the protein and consequently block amyloid fibril formation. Furthermore, tetracyclines can also interact with PrP aggregates and trigger their disassembly (Forloni et al., 2002; Tagliavini et al., 2000). Moreover, it was also found that tetracycline (tetracycline hydrochloride - doxycycline hyclate) treatment counteracts the protease resistance of PrP^{Sc} extracted from sporadic CJD brains and abolishes the deleterious effects of synthetic PrP peptides on cell survival and astrocyte proliferation (Forloni et al., 2002; Tagliavini et al., 2000).

Nevertheless, a phase 2, randomized, double-blind, placebo-controlled trial (Haik et al., 2014) found no significant effect of doxycycline on CJD patients. However, this study recruited

patients who had definitive or probable sporadic CJD or genetic forms of the disease; therefore, the accurate starting point for doxycycline therapy may have passed.

2.3.3 Anthracyclines

Finally, concerning the potential of antibiotics for the treatment of TSEs, it has been shown that the antibiotic DOX inhibits the amyloid pathway of PrP peptides, diminishes the infectivity of PrP^{Sc} from prion-infected brains and enhances survival in a Syrian hamster model of Prion disease (Forloni et al., 2009; Tagliavini et al., 1997). This finding is in good agreement with results from Corato and co-workers (2009) that indicate that the treatment of homogenates made from scrapie-infected brains with doxorubicin decreased its infectivity approximately ten thousand-fold, which indicates that the infective PrP^{Sc} form restrained within the homogenate is dramatically affected by the antibiotic.

2.4 Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a fatal and devastating neurodegenerative disorder that ultimately leads to the death of motor neurons; there is currently no cure (Hortle et al., 2016). This adult-onset disorder is characterized by progressive motor weakness with a focal beginning, which propagates across the body and inflicts paralysis and death usually within 2 to 5 years after diagnosis (Bunton-Stasyshyn et al., 2015; Hortle et al., 2016). Riluzole, a drug that blocks glutamatergic neurotransmission, is the only medication approved by the Food and Drug Administration for ALS. However, riluzole does not halt or prevent the disease but only slightly extends survival.

Despite the finding that the origin of ALS is usually sporadic, mutations in the gene that encodes for the enzyme superoxide dismutase (SOD1) have been linked to the disease (Bunton-Stasyshyn

et al., 2015). Furthermore, compelling evidence indicates that mutations in SOD1 induce the gain of toxic properties by the protein (Bunton-Stasyshyn et al., 2015; Kiernan et al., 2012; Saccon et al., 2013). Notably, it was found that SOD1 exhibits prion-like behavior *in vitro* and in cellular and animal models (Bunton-Stasyshyn et al., 2015; Chia et al., 2010). Moreover, SOD1 is capable of self-seeding and can undergo the amyloid fibrillization pathway (Bunton-Stasyshyn et al., 2015; Chia et al., 2010). Furthermore, it was found that spinal cord tissue homogenate from mice that carried the $SOD1^{G93A}$ mutation efficiently seed the amyloid aggregation of both wild type and mutant SOD1 *in vitro* (Bunton-Stasyshyn et al., 2015; Chia et al., 2010).

Interestingly, a few studies have reported the beneficial effects of antibiotics in models of ALS. In this sense, Kriz and co-workers (2002) found that minocycline retarded the motor-neuron degeneration and decelerated the progression of the disease in the mutant SOD^{G37R} mouse model of ALS when the antibiotic treatment began at a late presymptomatic stage. This finding is in agreement with results from Keller and colleagues (2011) that indicate that minocycline could slow the course of the disease in the GFAP-luc/SOD1G93A mouse model when administered during the presymptomatic stage. Conversely, when the medication was administered at a late stage of the disease, minocycline had no effect on the survival of animals, (Keller et al., 2011). Moreover, when minocycline was administered after the onset of the disease when microglia had been chronically activated, treated animals showed increased neuroinflammation and disturbed astrocyte reactivity (Orsucci et al., 2012).

Nonetheless, a randomized placebo-controlled phase III trial (ClinicalTrials.gov, number NCT00047723) could not replicate the effect observed in mouse models (Gordon et al., 2007). Instead, it was found that minocycline was ineffective in delaying or ameliorating the progression of the disease (Gordon et al., 2007). Furthermore, this study suggests that

minocycline may have a noxious effect on ALS patients, which may be due to an interaction with riluzole, as it appears to occur in the mouse model (Gordon et al., 2007; Orsucci et al., 2012). Notably, the concentration of minocycline used in this study was higher than that prescribed in current clinical therapy (Gordon et al., 2007).

On the other hand, evidence has arisen from many reports that suggests that altered autophagy may play a central role in ALS (de Paula et al., 2015; Hetz et al., 2009; Li et al., 2008), and several autophagy-enhancing compounds have been tested in ALS models (de Paula et al., 2015; Staats et al., 2013). For instance, the dietary administration of rapamycin, a well-known enhancer of autophagy, increased the expression of autophagy markers in a mutant SOD1 mouse model of ALS without extending their life span (Staats et al., 2013). However, when administered to mutant $SOD1^{G93A}$ mice that lacked mature lymphocytes, rapamycin triggered a mild increase in the survival of those animals, indicating that rapamycin may exert a dual action on disease progression. On one hand, it increases autophagy and the clearance of aggregates; on the other hand, rapamycin represses beneficial immune responses due to its immune suppressor activity (Staats et al., 2013). Nonetheless, Zhang and colleagues (2011) reported that in the mutant $SOD1^{G93A}$ mouse model, rapamycin mildly enhanced the aggregation rate of SOD1 in motor neurons and concomitantly accelerated the degeneration of those neurons. These dual and opposing effects highlight the need to develop rapamycin derivatives that lack immune-modulating effects while preserving the protective autophagy-inducer activity

Regarding ceftriaxone, it was found that the administration of this drug in a mouse model of ALS protects motors neurons from excitotoxicity and extends survival while retarding the decline of muscle strength and neuronal death (Rothstein et al., 2005). These effects were attributed to an up-regulation of the glutamate transporter GLT1 gene induced by the drug

(Cudkowicz et al., 2014; Melzer et al., 2008; Rothstein et al., 2005). However, in a combined phase 1, 2 and 3 clinical trial (ClinicalTrials.gov, number NCT00349622) to evaluate the efficacy of ceftriaxone in ALS, no significant differences in survival among the treated and control groups were found (Cudkowicz et al., 2014). The disappointing outcome from these clinical trials may again highlight the requirement of the accurate timing of drug administration in the course of the disease in order to maximize the benefits and reduce undesired effects.

Although a few reports suggest a protective role of certain antibiotics in ALS, most studies attributed the beneficial effects to actions on different processes during the course of the disease and did not study in detail the amyloid component of the disorder. Therefore, it would be worthy to analyze the action of all kinds of antibiotics that possess antiaggregating activity in ALS with a focus on SOD1.

To this end, we found that *de novo* amyloid fibers made from recombinant human α-synuclein could trigger the aggregation of native SOD1 in a cell-free extract of SH-SY5Y cells (unpublished data, not shown); these data agree with previous results (Koch et al., 2016). Interestingly, in this experimental paradigm, rifampicin could reduce the aggregation of SOD1 induced by α-synuclein fibers (unpublished data, not shown), which indicates a potential cross-talk between two proteins involved in different neurodegenerative diseases and demonstrates that rifampicin may be a valuable alternative for ALS.

2.5 A common structural feature against aggregation?

As described above, anti-aggregation activity has been demonstrated for small molecules that exhibit a wide variation in chemical formulas. Indeed, the structural diversity of the molecules described in this review is depicted in Figure 3. Moreover, it has been suggested that some of

these small molecules would target a common structural arrangement among proteins as diverse as PrP, tau, α-synuclein, and Aβ (Giorgetti et al., 2011). Considering these reports, one can infer that a common feature of small molecules could target amyloid aggregation. To search for a minimal common structure (MCS) among the antibiotics, we performed a pairwise comparison of the structures listed on the figure with the Similarity Toolbox in ChemMine (Backman et al., 2011). Surprisingly, all the structures contain the motif O=CCCOH or O=CCNH (highlighted in Fig. 3). This structural motif constitutes a proton donor/acceptor pair arrangement that is reminiscent of the motif present in the protein backbone. Since this motif is attached to a closed ring, it is arranged in an almost planar configuration at an average distance of 2.6 Å, similar to the one adopted by the protein backbone in the beta structure (Jahn et al 2010; Sunde et al., 1997a; Sunde et al., 1997b). We propose that this configuration might interfere with the formation of the cross-beta structure common to amyloid fibrils.

In the case of rifampicin, the presence of this structural motif is not clear. Rifampicin has been shown to acquire a zwitterionic form in solution as a consequence of proton transfer from an O(8)-H phenolic group to a secondary amine (Pyta et al., 2012). As a consequence, this oxygen could act as a proton acceptor. Moreover, Li and co-workers (2004) showed that the most active species responsible for the inhibition of α-synuclein fibrillation is an oxidation product of rifampicin rather than the antibiotic itself. Indeed, upon oxidation, the O(8)-H phenolic group is oxidized to a cetonic group, giving rise to the donor-acceptor pair as shown in figure 3.

The importance of this structural motif for the activity of tetracyclines against aggregation has also been suggested by Cosentino and colleagues (2008). Through a 3D-QSAR analysis on the effect of modified tetracyclines on PrP amyloidogenesis, they found that switching the keto-enolic group results in a decrease of activity. Moreover, the addition of hydroxyl groups on the

rings increases activity, presumably by providing donor sites for H-bond interactions with the protein.

Other structural features related to anti-amyloidogenic activity have been identified in other molecules, such as the presence of aromatic rings for stacking interactions (Cosentino et al., 2008; Pyta et al., 2012). This study does not rule out other structural features; on the contrary, we think that they could be related to other interactions within the aggregates that could offer some specificity. However, interference with the formation of the hydrogen bond on cross-beta structures should be essential in compounds that target aggregation.

Figure 3

3. Conclusions

Despite the evidence analyzed herein, some central questions remain unanswered, such as whether one compound could interfere an analogous process in related diseases, whether antibiotics with protein antiaggregating properties fulfill the requirements to be safely used in neurodegenerative diseases, and whether neuroprotective antibiotic therapy could increase the risk of dissemination of antibiotic-resistant pathogenic strains. According to data revised herein, we can try to answer these questions.

3.1 Canonical signature of neurodegeneration and the opportunity for multiple disorders intervention with antibiotics

Considering that the canonical signature and one of the first steps of the cascade of events leading to neurodegeneration is protein amyloid aggregation, it is currently accepted that toxicity

arises from soluble intermediates and from the end-point aggregates that elude proteasomal and autophagic routes (Gustot et al., 2015). Furthermore, neuronal impairment propagation is also caused by this abnormal protein aggregation, which is spread to neighbor neurons in a prion-like manner (Luk et al., 2012; Nath et al., 2012). In this scenario, a reduction in the toxicity of abnormal protein aggregation would be essential in the fight against neuronal death.

Notably, while the misfolded protein differs between AD, PD, and PrP-related disorders, the protein aggregation process appears to be mechanistically similar and appears to involve the same kinds of steps and interactions during the development of the pathological states. Therefore, it could be expected that one compound interfering a process, analogous between these disorders, would be capable of affecting those mechanistically related diseases. Interestingly, experimental proof indicates that antibiotics are suitable candidates for that mission. In fact, encouraging evidence suggests that the anti-aggregating action of some antibiotics is not restricted to one specific protein (Fig 4; Table 1). Tetracyclines, especially doxycycline, exert anti-aggregating activity on Aβ, α-synuclein, and PrP. Rifampicin, DOX and AmB exert anti-aggregating effects on different polypeptides that share as a common feature the ability to form amyloid aggregates. Moreover, in the present work, through a pairwise comparison of the structures of antibiotics with antiaggregating activity, we found a common structural signature in their molecules that could be of high relevance in rational drug design. In this regard, and according to Table 1, these antibiotics have been shown to confer neuroprotection against more than one disease, including AD, PD, and TSEs. Fig. 4 and Table 1 summarize the different steps in the protein aggregation process in which antibiotic interventions have been reported.

Figure 4

Table 1

3.2 Antibiotics as pleiotropic agents against common features of neurodegeneration

Compelling evidence indicates that most, if not all, neurodegenerative disorders involve oxidative and a neuroinflammatory processes which may contribute to degeneration and neuronal death (Chen et al., 2016; Gonzalez-Lizarraga et al., 2017).

An acute inflammatory response mediated by microglia, such as those observed in response to tissue damage or pathogen invasion, induces a self-limiting process through the immune system and promote tissue repair (Chen et al., 2016; Wyss-Coray and Mucke, 2002). However, when the inflammatory process became chronic dysregulated microglia activation leads to the release of proinflammatory mediators and neurotoxic factors which enhance neural damage (Edan et al., 2013; Roqué et al., 2016; Santa Cecilia et al., 2016). In this context, microglia, the resident macrophages of the central nervous system, has a dual behavior. On one hand it keeps brain homeostasis; on the other, it may strongly contribute to neuronal damage (Amor et al., 2010). Thereby, neuroinflammation plays a central role in the pathogenesis and progression of neurodegenerative disorders (Santa Cecilia et al., 2016). Interestingly, as mentioned above, aggregated proteins may induce a microglia mediated inflammatory response as well as oxidative damage. Moreover, evidence indicates that all these processes might be interrelated in a vicious circle in which protein aggregation induces neuroinflammation and oxidative stress and vice versa (Gonzalez-Lizárraga et al., 2017; Gustot et al., 2015; Zhang et al., 2000).

Notably, many antibiotics including b-lactams, tetracyclines and rifampicin, aside their antimicrobial activity showed antioxidant properties as well as a strong anti-inflammatory action

being capable of suppressing microglia activation, in both *in vitro* and *in vivo* models of neurodegenerative disorders (Bi et al., 2011; Lazzarini et al., 2013; Reglodi et al., 2017; Santa Cecilia et al., 2016; Wei et al., 2012). *i.e.*,minocycline and doxycycline block microglia activation through p38 MAPK signaling pathways, preventing the release of neurotoxic factors and pro-inflammatory mediators from activated microglia (Reglodi et al., 2017; Santa Cecilia et al., 2016).

Overall, neuroprotective therapy with antibiotics as multitarget pleitropic molecules capable of suppressing oxidative stress, microglia activation and protein aggregation, constitutes a promising therapeutic approach for halting the noxious cycle of degeneration at several levels.

3.3 Antibiotic therapy, human microbiota and the risk of resistance emergence

When used as antimicrobial agents, antibiotics may exert a selective pressure against bacterial species normally found in the human intestinal microbiota perturbing the symbiotic interaction microbia/human and therefore, affecting the physiologic processes that they are involved in (Jernberg et al., 2010). Morgun and colleagues (2015) found that antibiotics at antimicrobial doses may alter the gut through microbiome-dependent and independent processes. On one hand, normal microbiota depletion leads to local immunodeficiency; On the other, antibiotic's direct effects on host tissues as well as the colonization of the gut by remaining antibiotic-resistant microbes and opportunistic pathogens *i.e. Clostridium difficile*, result in epithelial cell death (Morgun et al., 2015; Langdon et al., 2016). Conversely, many reports indicate that treatments with antibiotics at low doses, below the minimal inhibitory concentration (MIC), do not perturb the microbia/human interaction neither kill the native commensal bacterial communities (Gu et al., 2012; Walker et al., 2005). Likewise, several clinical studies, some up to two years of

treatment, demonstrated the efficacy and safety of subantimicrobial-dose of doxycyline (SSD) (20 mg/day) in human patients suffering pathologies such as rheumatoid arthritis, type II diabetes, oral inflammatory and cardiovascular diseases (Gu et al., 2012; Walker et al., 2005). Moreover, concerning the risk of resistance development during treatment with subantibiotic-dose of doxycycline several clinical trials found no differences between placebo and SSD treatment regarding the native human microbiota, the emergence of antibiotic-resistant strains and colonization by opportunistic pathogens (Gu et al., 2012; Walker et al., 2005). Furthermore, compelling experimental evidence plainly show that long-term SDD treatment does not affect the normal human microbiota regardless whether it is in the colon, the vagina, subgingival or the skin (Ashley et al., 1999; Barnett et al., 2007; Giannobile et al., 2008; Gu et al., 2012). In addition, it is important to note that human microflora normally exists as bacterial biofilms whose antibiotic tolerance is significantly higher than those observed when microorganisms grow planktonically (Gu et al., 2012).

Overall, although more evidence is required, when used at subantibiotic concentrations antibiotic therapy appears to be safe without perturbing human microbiota communities. Interestingly, the US FDA has already approved two SSD formulations for human long-term therapy: Periostat, a systematically administered drug for the treatment of periodontal pathologies and Oracea, a novel sustained-release SDD formulation for the systemic treatment of rosacea, a chronic inflammatory skin disease (Gu et al., 2012)

3.4 Feasibility of antibiotic therapy against neurodegeneration

Regarding the feasibility of the use of antibiotic therapy in neurodegeneration, it is important to consider that, because of the BBB, neuronal disorders pose additional challenges; the pharmacologic approach may be particularly difficult and hinder the treatment. Therefore, a candidate molecule for drug therapy must be able to freely cross the BBB, or at least at a concentration high enough to exert its action. This is a crucial requirement since many potential drugs with established efficacy *in vitro* cannot efficiently cross the BBB, and they are unsuitable for *in vivo* therapy (Pardridge et al., 2005). Although alternative strategies for drug delivery are currently under study, and some of them exhibit relative success (Freese et al., 2014; Roney et al., 2005), it is desirable that the therapeutic agent reach the brain by itself without any requirement for additional compounds or delivery systems. In this sense, the fewer compounds involved in therapy, the lowest risk for patients.

Interestingly, many antibiotics with proven antiaggregating action are not only capable of efficiently crossing the BBB but also have a good safety record since they have been employed in human health for decades without serious secondary effects.

On the other hand, compelling evidence strongly suggest that many neurodegenerative disorders may share a common intestinal origin (Braak et al., 2006; Davies et al., 2006; Natale et al., 2011; Pan-Montojo et al., 2012; Shannon et al., 2012). Moreover, it has been reported that many disorders progress from the enteric nervous system through the vagus nerve by retrograde axonal transport of the pathogenic amyloid species, to finally reach the CNS (Natale et al., 2011; Pan-Montojo et al., 2012). In this context, oral antibiotic doses capable of reaching the brain at protective level but low enough to avoid microbiota disturbance may be also valuable in order to halt the formation and dissemination of toxic amyloid species within the intestine itself.

Notably, recent data suggest that Gram-negative bacterial molecules are associated with neurodegenerative pathologies, since LPS and *E coli* K99 *pili* protein levels are increased in the brains of AD patients compared to healthy individuals (Zhan et al., 2016). Thereby, although the antibacterial effect *per se* is not analyzed in this review, this concept strongly reinforces the idea that antibiotics are multifunctional and safe molecules that are ready to be tested in clinical trials to fight against neurodegenerative diseases.

Additionally, one essential point that should be carefully considered is the ratio between antibiotics and the aggregating protein concentrations. Most *in vitro* studies were performed with the molar ratio of 1:1 (antibiotic:protein) (Gonzalez Lizarraga et al., 2017; Tomiyama et al., 1994). Moreover, Umeda and colleagues (2016) found in a mouse model that lower concentrations of rifampicin are required for cognitive improvement in younger animals compared with older animals. Of note, antibiotics such as rifampicin and doxycycline reach the mammalian brain at concentrations in the order of μg/ml, as measured in the CSF, whereas the levels of amyloidogenic proteins are much lower, in the range of ng/ml (Dorey et al., 2015; Mindermann et al., 1993; Mollenhauer et al., 2011; Tomiyama et al., 1994; Yim et al., 1985). Therefore, considering the level achieved in the brain, doxycycline and rifampicin might exert antiaggregating action at sub-antibiotic doses *in vivo* without imposing selective pressure on human microbial populations.

Overall, the capacity of behaving as pleiotropic molecules targeting multiple features of neurodegeneration, the lack of serious side effects, and the fact that the concentrations required to reach the brain and to exert protective action are subantibiotic and harmless for native microbiota led us to propose doxycycline, and in a less extention rifampicin, as ready-to-use molecules against neurodegenerative processes.

Finally, concerning the administration time of the drugs, since these disabling disorders involve the death of neurons and thus once they are lost the brain damage becomes irreversible, therapeutic strategies should mainly be focused in blocking the first steps of the cascade of events leading to neurodegeneration. Likewise, since protein aggregation is one of the first events of that deleterious cascade, antibiotics that block the amyloid pathway would be more adequate for the early stage of the diseases although they may also be beneficial at later stages.

Nevertheless, it should be noted that the difficulty for early accurate diagnosis may interfere with the efficacy of therapy, and unfortunately, the appropriate starting points for treatment may have passed. In this regards, not only effective drugs but also trustworthy diagnosis methods are strongly required in order to eradicate or at least slowing down the incidence of disabling disorders such as AD, PD and TSEs.

Be that it may, despite the long history of antibiotic in human health, they have arisen as a new encouraging alternative against amyloid-associated diseases, expected to play a central role in the fight against neurodegenerative disorders, the most challenging epidemic of the 21st century.

Conflict of interest statement

The authors have no conflicts of interest.

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Figure 1. Schematic representation of the aggregation pathways. (A) Upper-panel: Within a neuron, native proteins undergo structural modifications and become prone to aggregation. Depending on environmental conditions, aggregation leads to the formation of either off-pathway or on-pathway species. Off-pathway species do not evolve into fibrils. Conversely, on-pathway intermediates undergo the amyloid process to finally form insoluble fibrils. Lower-panel: On-pathway species released from diseased cells reach neighbor neurons and trigger the conversion of native protein into more on-pathway pathological species. (B) Aggregation kinetics are markedly affected by seeds made of on-pathway species that significantly accelerate the amyloid aggregation process.

Figure 2. (A) Putative structural model of gramicidin S (orange) bound to amyloid beta peptide fibrils (cartoon representation in lime). The side chains of Phe19, Asp23 and Lys28 from amyloid β-peptide are represented explicitly in licorice, as well as the sidechains of ornithine and D-Phe from gramicidin. Gramicidin has been proposed to bind to an amphipathic channel formed within the hairpin like structure of the fibril. (B) Licorice representation of the crystallographic structure of gramicidin S. Ornithine has been proposed to interact with Asp23, debilitating the intermolecular salt bridge formed between Asp23 and Lys28 from adjacent subunits. D-Phe

would form π-stacking interactions with Phe19, while Val and Leu sidechains would interact with the hydrophobic surface of the amyloid β-sheet

Figure 3. Chemical structures of the antibiotics with anti-amyloidogenic activity analyzed in this review. The blue boxes highlight a common structural motif among them. Oxidation of rifampicin to rifampicin quinone leads to the formation of the common structural motif which correlates with an increased effect on protein aggregation.

Figure 4. Effects of antibiotics on the amyloid aggregation pathway of different disease-associated proteins. The actions of antibiotics at several levels on the noxious amyloid pathway of a) α-synuclein (Parkinson's disease, synucleinopathies), b) Aβ (Alzheimer's disease), c) Tau (Alzheimer's disease) and d) Prpc (Transmissible Spongiform Encephalopathies) are schematically represented.

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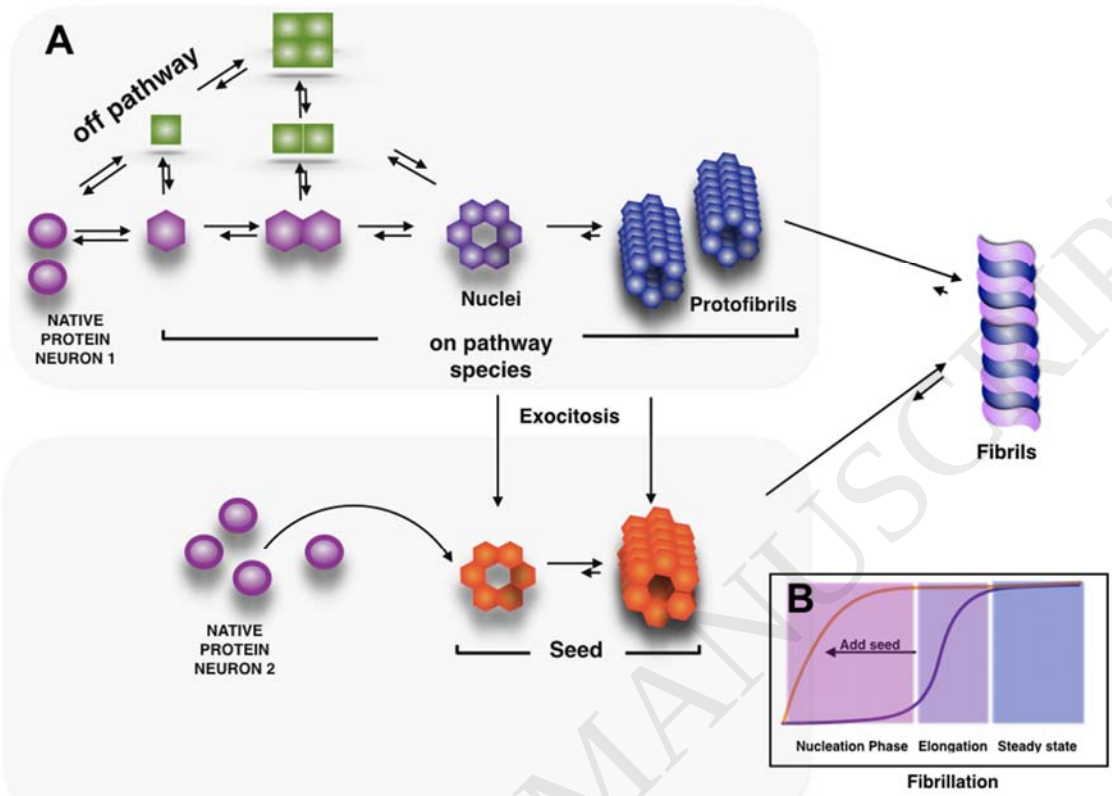

Figure 1
Authors: Chehin, R.N., Socias, S.B., Avila, C.L.
Progress in Neurobiology

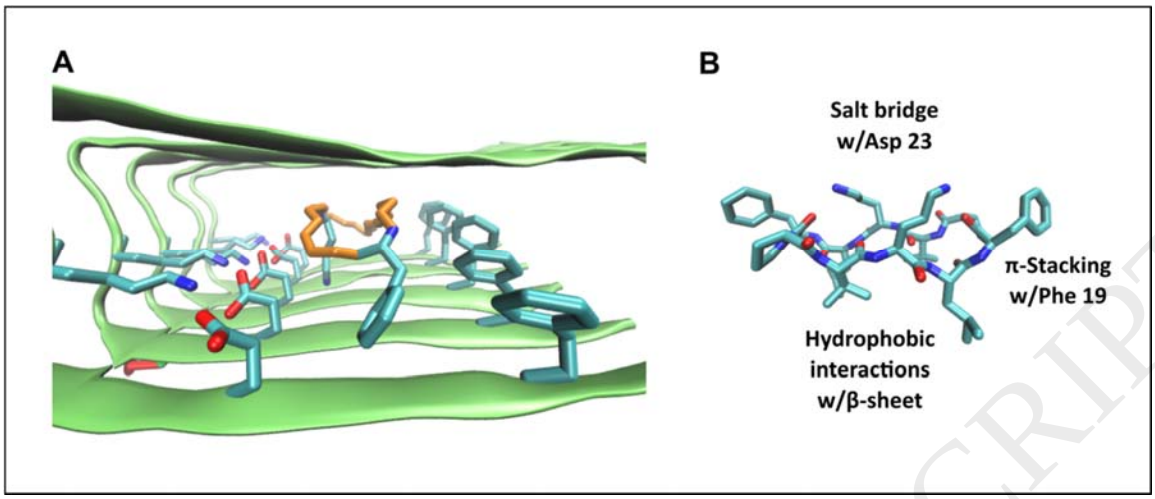

Figure 2
Authors: Chehin, R.N., Socias, S.B., Avila, C.L.
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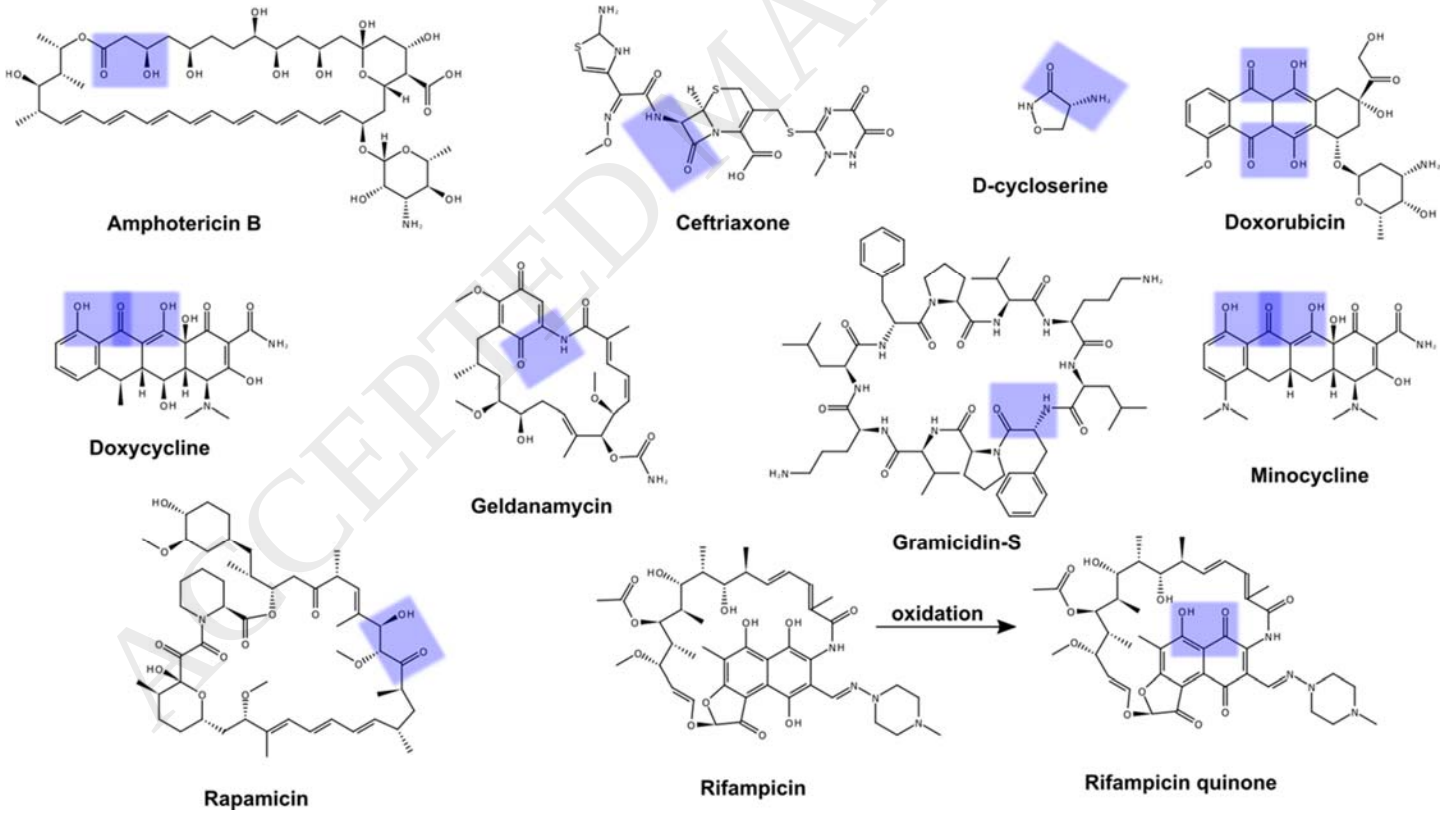

Figure 3

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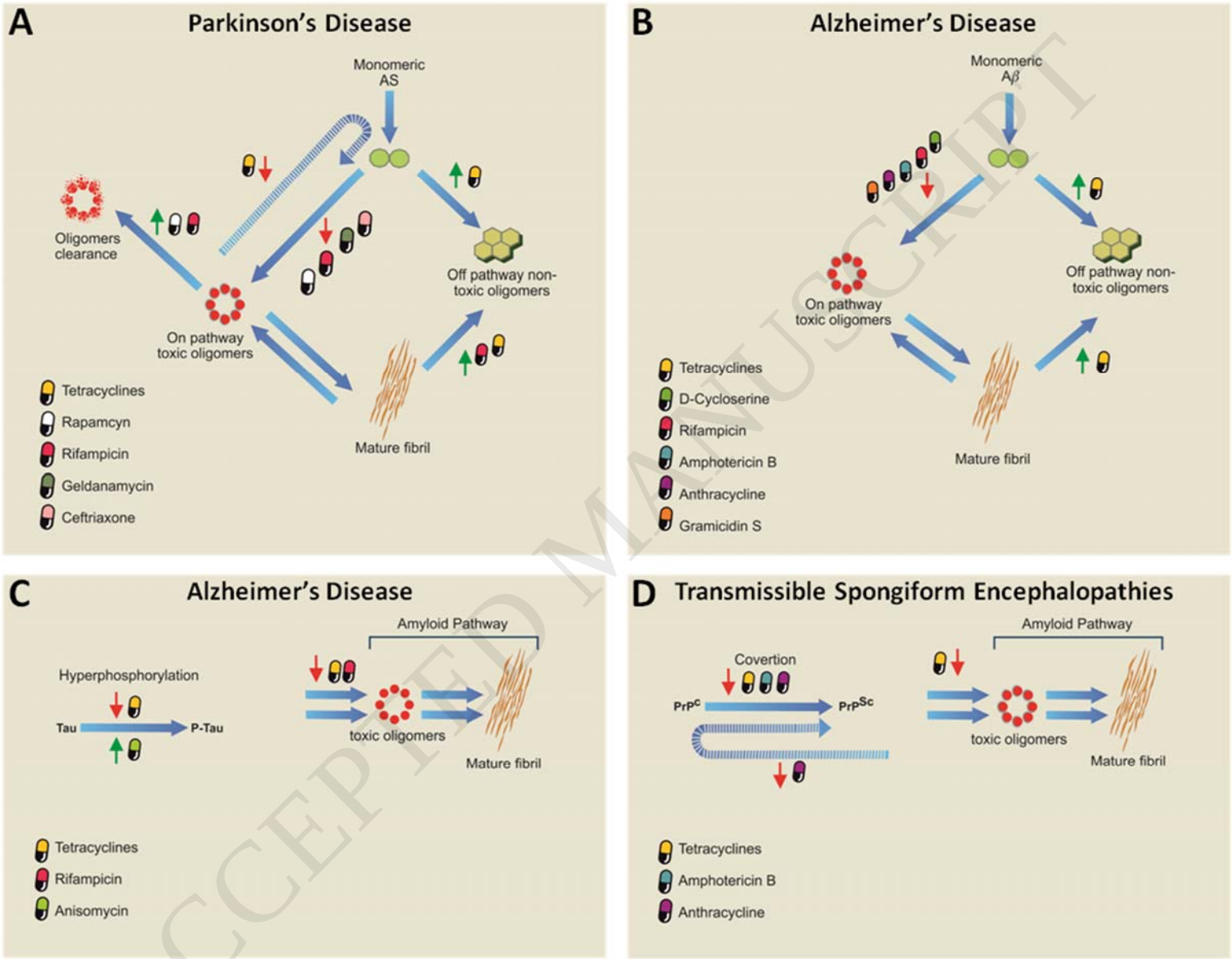

Figure 4
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Antibiotic	Alzheimer's Disease	Parkinson's Disease	PrP-related Diseases	References	Clinical Trials
Tetracyclines	• Inhibit the Aβ amyloid fibrillization pathway • Lead to the building of non toxic amorphous soluble aggregates • Reduce the formation of tau aggregates	• Block the α-synuclein amyloid fibrillization pathway • Lead to the building of non-toxic amorphous soluble aggregates • Block the seeding effects of amyloid aggregates on native α-synuclein	• Inhibit the conversion of PrP^{C} into the pathological form PrP^{Sc}. • Disassemble PrP-aggregates	• AD: Diomede et al. 2010; Forloni et al. 2001; Airoldi et al. 2011; Costa et al. 2011 • PD: González-Lizárraga et al. 2017; Ono and Yamada 2006 • PrP-rD: Forloni et al. 2002; Forloni et al. 2013; Tagliavini et al. 2000	• NCT00715858; NCT00692588; NCT00439166; NCT00355576; NCT00063193; NCT01463384; NCT00029874; NCT00047723; NCT00277355; EudraCT 2006-001858-27 / 2007-005553-34
Gramicidin S	• Blocks the Aβ aggregation pathway *in vitro* • Disassembles pre-formed Aβ amyloid fibrils			• Luo et al. 2013	
D-Cycloserine	• Diminishes Aβ-42 fibrillization			• Chaturvedi et al. 2015	
Amphotericin B	• Diminishes Aβ-42 fibrillization		• Inhibits the conversion of PrP^{C} into the pathological form PrP^{Sc}	• AD: Hartsel et al. 2003 • PrP-rD: Xi et al. 1992; Mangé et al. 2000ab; Cronier et al. 2007; Soler et al. 2008	
DOX	• Decelerates the Aβ fibrillization process		• Inhibits the PrP amyloid pathway • Diminishes the infectivity of PrP^{Sc}	• AD: Merlini et al. 1995 • PrP-rD: Tagliavini et al. 1997; Forloni et al. 2009; Corato et al. 2009	
Anisomycin	• Increases the level of the aggregation-prone pathogenic hyperphosphorylated tau			• Wang et al. 2008	
Rifampicin	• Inhibits the aggregation process of Aβ1-40 *in vitro* • Decreases the accumulation of Aβ and tau oligomers and reduces tau hyperphosphorylation	• Inhibits of the aggregation pathway of α-synuclein. • Disassembles α-synuclein pre-formed fibrils • Promotes the removal of misfolded α-synuclein through GRP78		• AD: Tomiyama et al. 1994; Tomiyama et al. 1996; Tomiyama et al. 1997; Umeda et al. 2016 • PD: Li et al. 2004; Xu et al. 2007; Jing et al. 2014	• NCT00715858; NCT00439166; NCT01002079; NCT00692588
Geldanamycin		• Decreases α-synuclein aggregation level through up-regulation of Hsp70		• Aulucket al. 2002; McLean et al. 2004	
Ceftriaxone		• Inhibits the α-synuclein amyloid aggregation pathway		• Ruzza et al. 2014	• NCT00349622; NCT00718393
Rapamycin		• Diminishes α-synuclein aggregation • Enhances oligomers clearance through increased autophagy		• Liu et al. 2013	

Table 1. Effects of antibiotics on the amyloid aggregation pathway of disease-related proteins