Could Antibiotics Be Therapeutic Agents in Alzheimer's Disease?

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Abstract: Alzheimer´s disease (AD) is an irreversible neurodegenerative disorder and one of the main aging-dependent maladies of the 21st century. Around 46 million people suffer from AD worldwide and this is projected to double within the next 20 years. Due to the progressive aging of the population and the prediction of an increase in the incidence of this disease, AD constitutes a serious familial and social health problem. Therefore, it is necessary to find new therapeutic strategies which are aimed to prevent, delay the onset, slow the progression and/or improve the symptoms of AD. Currently, the research is focused on finding and identifying new drugs for achieving these goals.

In this chapter of the book, we widely review the neuroprotective role that some antibiotics could play in AD, because these drugs reach the brain quickly and are relatively inexpensive. Likewise, we have found evidence in both *in vitro* and *in vivo* studies and also in some clinical trials. In summary, all the reviewed antibiotics exert neuroprotection because they act on the main pathophysiological features of AD. Nevertheless, it must be taken into account that a long-term treatment with antibiotics could cause adverse effects including antibiotic resistance. Thus, properly clinical trials should be carried out in order to corroborate benefits of these antibiotics in people with AD.

Keywords: Alzheimer´s Disease, Amphotericin B, Amyloid β, Antibiotics, Azithromycin, Clioquinol, Dapsone, Doxycycline, Minocycline, Tetracycline Rapamycin, Rifampicin.

INTRODUCTION

Alzheimer´s disease (AD) is the most common cause of dementia worldwide. Currently, around 46 million people suffer from AD, and these data will be duplicated in 20 years. The aetiology of the disease is multifactorial (genetic, environmental, behavioural…); nevertheless, the greatest risk factor is aging. The global demographic trend indicates that population aging is quickly increasing. The World Health Organization estimates that, by 2040, the proportion of world population aged ≥65 will be 1.3 billion, 14% of the total). Due to the progressive aging of the population and to the prediction of an increase in the incidence of this disease, AD constitutes a serious familial and social health problem. In 2015, direct medical costs, social costs and the cost of informal care added up to a total of $818 billion (US) at the global level. Therefore, it is essential to find therapeutic strategies which are designed to prevent, delay the onset, slow the progression and /or improve the symptoms of AD. Nowadays, the lines of research focus on finding and identifying new drugs to address these issues. In this chapter of the book, we have focused on a thorough review of the neuroprotective role of the antibiotics rifampicin, rapamycin and minocycline as well as other antibiotics, such as azithromycin, erythromycin, clioquinol, amphotericin B and tetracycline, in order to see what their role may be in the treatment of AD as these compounds reach the brain quickly and are relatively inexpensive. Likewise, we have deeply analysed both *in vitro* and *in vivo* studies and clinical trials, in order to explain the possible action mechanisms of these drugs and examining their possible clinical use.

PATHOPHYSIOLOGY OF AD

AD is a neurodegenerative disorder which induces progressive memory loss and cognitive decline, exacerbated by neurotransmitter deficits. The pathophysiology

of the disease is complex. The presence of extracellular senile plaques containing amyloid beta peptide (Aβ) and intracellular neurofibrillary tangles (NFTs) of hyperphosphorylated tau protein are neuropathological characteristics in brain of people with AD [1, 2].

The “amyloid theory”, which is based on the over expression and aggregation of Aβ, is believed to be one of the main causes of its aetiology [1 - 8].

The amyloid protein precursor (APP) can normally be cleaved by α-secretase and γ-secretase (the non-amyloidogenic pathway) or can aberrantly be processed by β-secretases and γ-secretase (the amyloidogenic pathway), leading to the production of Aβ (Fig. **1**). As a consequence, Aβ peptides spontaneously aggregate into soluble oligomers and combine to form fibrils insoluble beta-sheet conformation and are eventually deposited in diffuse senile plaques resulting in an imbalance between production and clearance of Aβ peptide [2, 3].

APP is a type 1 transmembrane glycoprotein consisting of a long N-terminal extracellular segment (ectodomain), a transmembrane domain and a shorter intracellular C-terminal portion (the cytoplasmic domain). Its expression is mainly localized around the synapse of neuronal tissue. The gene encoding APP is located in chromosome 21. Although its primary role is not fully understood, it is crucial for neuronal plasticity and synapse formation.

The primary proteolytic events on APP, whether pro- or anti-amyloid, occur at or around its transmembrane region.

The APP cleavage site for α-secretase is very close to the cell membrane surface and gives a soluble extracellular APP fragment (sAPPα) and an 83 amino acid, membrane-bound, carboxy terminus fragment (C83). Following intramembrane proteolytic cleavage of C83 by γ-secretase releases a short extracellular p3-peptide (p3) and a cytosolic APP intracellular domain (AICD). The α-APPs fragment has been described having neurotrophic and/or neuroprotective effects and that the AICD has nuclear signalling functions.

Cleavage of APP by β-secretase 1 (also named beta-site amyloid precursor protein cleaving enzyme 1 or BACE1) produces a soluble extracellular fragment (sAPPβ) and a 99 amino acid, membrane-bound, carboxy terminus fragment (C99). Subsequent cleavage of C99 within its transmembrane domain by γ-secretase releases extracellular Aβ and an AICD [4 - 6].

γ-Secretase is a multiprotein complex consisting of Presenilin1 (PS1), nicastrin, Aph-1, and PS2, and all four proteins are necessary for full proteolytic activity [7]. Together with APP gene mutation, several mutations in PS1 and PS2 genes have been described to be involved in familial AD [5].

As previously explained, the level of Aβ in the brain is controlled by its production and clearance. A chronic imbalance between these two processes may result in an accumulation of Aβ in the brain.

The Aβ species are substrates for various proteases. The most studied are neprilysin, that degrades monomeric and oligomeric forms of Aβ, and the insulindegrading enzyme (IDE), selective for monomers. Other proteases have been implicated in Aβ degradation including endothelin-converting enzyme (ECE-1), which is also selective for monomers, plasmin, that cleaves monomers and fibrils, angiotensin-converting enzyme (ACE) and the cysteine protease cathepsin B [4, 8].

On the other hand, one of the hypotheses is that increased Aβ level is a result of its faulty clearance across the blood-brain barrier (BBB). Aβ clearance across the

BBB is mediated by receptor(s) or transporter(s) such as the low density lipoprotein receptor-related protein-1 (LRP1), P-glycoprotein (P-gp) and the multidrug resistance-associated protein 1 (MRP1) [9].

An increasing number of studies suggest that both alteration of expression and functional activity of LRP1 and P-gp contribute to the accumulation of Aβ in the brain and lead to increased risk for developing AD [10]. Moreover, recent evidence indicates a progressive decline in the levels of LRP1 and P-gp at the BBB during normal aging and this decline was positively correlated with accumulation of Aβ in AD [11].

Aβ aggregations are tightly linked to increased oxidative stress, which is accompanied by mitochondrial dysfunction, pronounced inflammation, gliosis, axonal degeneration and impairment of synaptic transmission induced by the deregulated cellular proteostasis [12], which ultimately ends in progressive neuronal loss, predominantly by apoptosis [13]. Even the impaired phagocytic activity of microglia favours the Aβ deposition, exacerbating memory loss [14].

The other major hallmark of AD are the intracellular NFTs [1, 2]. The microtubule associated protein tau, the main constituent of NFTs, is normally synthesized by neuronal cells in order to stabilize the microtubules for proper functioning of the neurons, particularly axonal morphology, growth, and polarity [6]. The activity of the protein tau is mainly regulated by phosphorylation. Tau hyperphosphorylation is thought to result from an imbalance in the function of several protein kinases and phosphatases [15]. Increased phosphorylation of tau, destabilizes tau-microtubule interactions,leading to microtubule instability, transport defects along microtubules, and ultimately neuronal death. Hyperphosphorylated tau detached from microtubules and becomes mislocalized from the axon to the neuronal soma and dendrites and forms this abnormal filaments [1, 2, 6].

Neuroinflammation

As one of its many features, AD is characterized by an Aβ-induced chronic inflammatory state, Aβ peptides and their aggregations can induce an inflammatory reaction that subsequently triggers cognitive decline and the development of neurodegeneration in the brain of people with AD [16]. Microglia and astrocytes are the main cellular players in this state because they release cytokines, interleukins and reactive oxygen species after exposure to Aβ, thereby exacerbating a neuroinflammatory reaction [17, 18]. Up-regulation of proinflammatory mediator genes expression was observed in Aβ-treated primary cultures of human microglia [19]. An increased Aβ load in aging TgAPPsw and PSAPP transgenic mice is associated with an increased production of inflammatory cytokines as well as an adjacent neuronal death by releasing reactive oxygen intermediators and proteolytic enzymes [20, 21]. Nevertheless, AD-associated inflammation is named “cytokine vicious cycle”, because the generation of Aβ, from amyloidogenic processing of APP, is enhanced by cytokines and interleukins, such as TNF-α, IL-6 and IL-1β. At the same time, Aβ is able to stimulate a nuclear factor kappaB- (NFκB) dependent pathway that is required for cytokine microglial production [22, 23]. Microglia, the guardian phagocyte of CNS, is responsible for initiating an innate immune response through pattern recognition receptors (PRRs) that bind to danger-associated molecular patterns (DAMPs) from pathological triggers. DMAPs can be released from either extracellular or intracellular space after injury. During the course of AD, the most frequent DAMPs is Aβ both in oligomeric form and fibrils or

aggregates [24]. Different Aβ forms can bind to cell-surface microglial receptors, including SCARA1, CD36, CD14, CD47 and Toll-like receptors (TLR) [25, 26]. It has been reported that TLR2 and human Aβ-triggered microglial activation is through NFkB-dependent pathway. Moreover, TLR4 can trigger the Aβ-induced activation of murine microglia [27, 28]. So, the microglial binding between Aβ and TLRs actively promotes the neuroinflammation in the brain of people with AD. In the same way, deletion of CD36, TLR4 and TLR6 *in vitro* decreases Aβ-induced cytokine production and prevents amyloid accumulation [29]. In fact, microglia aids in Aβ clearance mechanisms, internalizing phagocytosis-mediated soluble Aβ fragments, which are deleted by neprilysin and IDE. However, in chronic inflammation conditions the microglial phagocytic capacity is impaired by fibrillar Aβ. *In vitro* experiments have shown that microglial phagocytosis is regulated by proinflammatory cytokines [30, 31]. In addition, a study carried out in humans, demonstrated that microglia deteriorate with age, reducing its phagocytic property [32]. In consequence, this insufficient microglial phagocytic capacity induces Aβ pathological accumulation, exacerbating the neurodegeneration in AD.

Astrocytes are also involved in the AD-associated inflammatory response. Like microglial cells, astrocytes produce inflammatory mediators through a limited set of PRRs which bind to Aβ [33]. Additionally, astrocytes degrade Aβ fragments and may promote the Aβ clearance in healthy adult mice [34]. Conversely, under an Aβ-induced chronic inflammation, astrocytes become reactive, expressing high levels of GFAP with ensuing astrocytic hypertrophy and proliferation [35]. Reactive astrocytes have been observed in the hippocampus and cerebral cortex in the majority of transgenic mice models with AD, increasing the pathogenesis of this neurodegenerative disease [36]. Therefore, depending on the levels of inflammatory mediators present in the environment, astrocytes may mediate a beneficial or harmful effect on AD.

The cytokines, including TNF-α, IL-1β, Il-6 and IL-4, are the main inflammatory mediators involved in AD-associated neuroinflammation. Numerous studies have reported high levels of these proinflammatory cytokines in the brains of transgenic mouse model and people with AD reducing the concentration of antiinflammatory cytokines, such as IL-4 [37, 38]. An *in vitro* study reported that IL-1β can boost Aβ aggregation by favouring amyloidogenic APP processing [38]. IL-1β and IL-6 seem to be important molecular mediators of astrocytic activity [35]. Likewise, TNF-α and Il-1β reduced Aβ phagocytosis and might impair neuronal function by the secretion and production of neurotoxic reactive oxygen species [30, 39]. Conversely, the transgenic expression of IL-1β in APP/PS1 mice led to a beneficial form of inflammation since Aβ aggregation decreased [40]. Therefore, some proinflammatory cytokines could be considered helpful in reducing AD like pathology in transgenic mouse models.

Finally, the role of tau protein in neuroinflammation should be considered. Tau transgenic mice showed microglial activation, synapse loss and impaired neurologic function [41]. Recently, it has been observed that a potentiated neurodegeneration and neuroinflammation in transgenic mice expressing tau mutant mediated by recruiting proinflammatory monocytes and enhancing sensitivity to neurotoxicity [42].

Autophagy

Proteinopathies are neurodegenerative disorders in which aggregated proteins are

abnormally accumulated. AD involves different protein accumulation and based on the evidences many researchers classified AD as a proteinopathy. It is characterized as a dysfunction in the protein homeostasis control processes including autophagy. There is much evidence demonstrating a relationship between autophagic alteration in normal aging and AD [43]. Moreover, it has recently been proposed that the gender differences in the risk of AD are related to differences in autophagy activity throughout the person´s life. The lowering levels of estrogen in women during menopause may contribute to an increase AD risk compared to men [44].

Autophagy is a catabolic, physiological, intracellular and ubiquitous process by which old, damaged or misfolded aberrant proteins and/or organelles are degraded in lysosomes, so that cellular components are recycled. Only the proteins with a specific sequence can be degraded in lysosomes [45, 46]. There is overwhelming evidence demonstrating the important neuroprotective role exerted by autophagy and the existing relationship between defective autophagy and the development of different neuropathologies including Parkinson´s disease, Huntington´s disease and AD [47 - 50]. This process is crucial in maintaining cellular homeostasis and the proper function of cells in many organs, including the brain [43, 51, 52]. It plays an important role in controlling the quality of cellular protein and organelles which could otherwise be harmful to the cell. Autophagy also plays important roles when nutrients are insufficient, in embryonic development, pathogen defense and even, tumor protection [53, 54]. There are three major subtypes of autophagy: microautophagy, chaperone-mediated autophagy and macroautophagy. While macro-and microautophagy involve the “in bulk” degradation of regions of the cytosol, chaperone-mediated autophagy is a more selective pathway and only proteins with a lysosomal targeting sequence are degraded [55]. Macroautophagy is the most frequently used process for recycling proteins and organelles, being linked most to AD and other neuropathies. This is a multiple step process consisting of sequential steps including sequestration, by which the cytoplasmic components or organelles are firstly surrounded by a simple membrane; phagophore. This structure elongates forming the autophagosome constituted by a double or multi-membrane, forming a cytosolic vesicle; autophagosome. In this process the material is sequestrated, but to be degraded it must be transported to lysosomes. Autophagosome fuses with a lysosome forming an autophagolysosome and then, material is degraded by hydrolases. Finally, amino acids, lipids, nutrients and metabolites are reused [56]. At least 30 proteins are involved in the process of autophagosome formation [57]. Atg1 was the first such protein identified and shown to have intrinsic serine/threonine kinase activity, which is essential for the initiation of autophagy [58]. Autophagy is regulated by phosphatidylinositol 3-kinase (PI3K) type I and type III. PI3K type I is activated by growth factors and suppresses autophagy through the regulation of the mammalian target of rapamycin (mTOR). One of them is Beclin 1 (Atg6), which constitutes a complex in which PI3K III is included and plays an essential role in omegasome formation (lipid bilayer membranes). The expansion of these membranes depends on two pathways; the Atg5–Atg12 pathway and the microtubule-associated protein 1 light chain 3 (LC3) pathway [59]. Soluble LC3 is translocated to autophagic membranes and Phosphatidylethanolamine is ubiquitin-like conjugated to LC3. This mechanism involved in the named conventional autophagy. The levels of Beclin-1 are reduced in both in humans and AD mouse models which are related to a decrease

in Aβ clearance [60, 61]. On the contrary, when this protein is up regulated after the injection of a lentivirus, autophagosoma formation and Aβ clearance are increased. Finally, an alternative autophagy model has been proposed, which involves vesicles from trans-Golgi and late endosomes. In this process, Rab 9 attaches to autophagic membranes after Unc51-like (ULK) activation [57] (Fig. **2**).

As it has been described, autophagosome biosynthesis impairment is related to Aβ accumulation, but it is also important to highlight the relationship between autophagosome formation and neurofibrillary accumulation. The function of tau protein is related to the normal assembly of microtubules. In AD, the deposits of neurofibrillary tangles alter cytoskeleton activity, which then may alter the autophagosome formation and fusion with lysosomes [62]. Moreover, impairment of autophagolysosomes formation may contribute to tau protein aggregates accumulation and cytotoxicity [63]. This alteration in the autophagolysosoma formation induces cellular dysfunction and death [63, 64]. This was demonstrated by using modified human neuroblastoma cells which express human tau, used as a model of taupathy which were treated with chroriquine [65].

Many other proteins have been involved in this process. It is important to highlight the role of some kinases such Atg1, which exerts serine/threonine kinase activity and initiates autophagy [58]. When PI3K type I is activated, it inhibits autophagy through mTOR that phosphorylate and inhibits ULK. In AD, mTOR overactivation seems to be implicated in the alteration of autophagosome synthesis through the inhibition of kinases such as ULK [66, 67] but also LC3 [68]. Likewise, the role of this kinase is described in more detail in the antibiotic section (rapamycin). Finally, the AMP-activated protein kinase (AMPK) is another kinase which is also phosphorylated and inhibits ULK and blocks autophagy in consequence.

In summary, it has been convincingly demonstrated that autophagy promotes a neuroprotective effect. In fact, there are defects in the autophagic course in many neural diseases, which is the case with AD [47]. Any alteration of this process, formation or function, causes protein aggregation and many studies are focused on the development of new therapeutic drugs which increase autophagosoma formation in AD.

Oxidative Stress

Diverse studies have found that Aβ toxic properties are mediated by different mechanisms and one of them is oxidative stress. However, the exact role of Aβ oligomers in this mechanism is widely debated [69]. The paramagnetic electronic resonance, a high-sensitive method for direct detection of free radicals, has been used to examine the pro-oxidant effect of Aβ [70, 71]. To observe this effect, high doses of the peptide are normally requested but the concrete mechanism is still unknown. Aβ has several metal-binding sites located in its first 15 amino acids constituted by the tyrosine 10 and the histidines 6, 13 and 14 position. These wellknown and powerful metal-binding sites have a high affinity for Cu^{2+} as well as for other metallic chelators [72]. Furthermore, Cu^{2+} can be bound by the nitrogen atoms located in the histidines´ imidazole rings, suggesting that the oxygen, which is necessary to enable this binding, can be provided by multiple groups, like the carboxylated lateral chain of the glutamate 5, the hydroxyl group of the tyrosine 10 or the ending amino group as well as from a water molecule [73].

Aβ has the capacity of reducing Cu^{2+} and Fe^{3+} to Cu^{+} and Fe^{2+}, respectively. Thus, the O_2 can react with reduced metals generating superoxide anion which combines

with two hydrogen atoms to form hydrogen peroxide that could react with another reduced metallic ion and forming the hydroxyl radical by Fenton-Haber Weiss reaction. The radical form of Aβ can extract protons from close lipids all proteins generating lipid peroxides (precursors of 4-hydroxynonenal which affects cellular signal transduction) and carbonyls, respectively [73, 74]. It has also been shown that these metals´ reductions are mediated by a 35 position-located methionine, whose sulfide group has the ability to oxide and donate electrons. Moreover, several studies have confirmed that when this amino acid is substituted, Aβ´s oxidative properties totally disappear [75]. However, the role of this residue is not entirely clear due to the fact that the oxidation of neurotransmitters exposed to Aβ´s 1-16 and 1-12 peptides bound to metal but lacking of Met35, which has previously been confirmed [76]. On the other hand, Aβ can trigger mitochondrial dysfunction owing to the fact that it has been found in different intracellular structures like the mitochondria´s inner membrane or matrix. Aβ can directly inhibit the generation of mitochondrial ATP affecting the correct action of a subunit of ATP synthase. Furthermore, Ab administration in subtoxic doses and in a chronic manner can inhibit the transportation of some nuclear proteins to the mitochondria. Therefore, Aβ is able to cause changes in mitochondrial permeability with the consequent release of cytochrome C and because of this, the apoptosis is activated [71]. Additionally, an increased expression of the divalent metal transporter 1 has been seen in the animal model APP/SS1 transgenic mouse, in the senile plaques of people with AD and in cellular lines which overexpressing APP, all of them being related to higher levels of iron in human cells exposed to Aβ, suggesting that impairments in iron homeostasis could induce an increment in oxidative stress caused by Aβ [77].

ANTIBIOTICS

Current AD pharmacologic therapy is aimed at treating the cognitive symptoms but do not alter the course of the disease. Cholinesterase inhibitors (rivastigmine, galantamine, donepezil) and memantine (a NMDA antagonist) are the only drugs approved for its treatment. Nonetheless, there is a wide range of components with a different nature and therapeutic purpose, such as antibiotic, antipsychotic and antihypertensive, that exert assorted neuroprotective effects in AD [2, 78].

In the literature there are many studies both pre-clinical and clinical which demonstrate that these candidates may interact with AD-associated pathophysiological mechanisms, inducing beneficial effects. Even recent studies have attributed neuroprotective properties to some foods such as extra virgin olive oil (hydroxytyrosol) [79], grapes (resveratrol) [80], fresh fish (omega 3 fatty acids) [81] and beverages such as green tea [81] and coffee [82].

Here, we review the known neuroprotective effects of some antibiotics on AD development because these inexpensive and interesting candidates are able to cross the BBB and by reaching the brain, target organ of this disorder [83, 84]. Currently, the rifampicin, rapamycin and minocycline are the most common antibiotics used both in preclinical and clinical studies, so in this review, we will bring the neuroprotective role of these antibiotics on AD up to date. Also, we will briefly update the actions of other, lesser studied antibiotics.

Dapsone, Rifampicin and Doxycycline

Dapsone, is a synthetic derivative of diamino-sulfone and was the first antibiotic to be widely used for leprosy. Also, it has shown significant anti-inflammatory activity as an inhibitor of the enzyme myeloperoxidase (MPO) in neutrophils and inhibits the integrin-mediated adherence and chemotaxis of them. Thus, it has

been used to treat a number of allergic and autoimmune disorders [85].
Rifampicin, also known as rifampin, is a semisynthetic antibiotic derivative of rifamycin and synthesized by the bacterium *Amycolatopsis rifamycinica*. Rifamycins are a subclass of the larger family of ansamycins. The rifamycins' mechanism of action is to selectively inhibit bacterial DNA-dependent RNA polymerase and show no cross-resistance with other antibiotics in clinical use. Rifampicin is an antibiotic with a very broad spectrum of activity and is used in the treatment of mycobacterium infections, including tuberculosis and leprosy [86, 87]. However, rifampicin and other rifamycins, as well as dapsone, are typically used in combination with other antibacterial drugs to slow or prevent development of resistance. Hepatotoxicity is generally rare alone, but preexisting conditions can be exacerbated. Rifampicin is lipid-soluble and following oral administration, it is rapidly absorbed and diffuses well to most body tissues and fluids, as well as to the brain by crossing the BBB [87].
Apart from their antimicrobial effects, rifampicin and dapsone, between other anti-leprosy drugs, seem to have additional properties that could have an impact on AD [85 - 93]. Thus, an epidemiological study showed that in Japan, a group of patients with leprosy treated with anti-leprosy drugs, had a significantly lower incidence of dementia compared with an untreated group [88]. In addition, histological analyses indicated that elderly non-demented leprosy patients in Japan showed significantly lower levels of amyloid plaques in the brain than nondemented,
non-leprosy subjects, whereas the number of NFTs where either
unchanged or increased [89, 90]. However, other studies did not confirm these results in the brain samples analyzed [91, 92]. Due to the controversy whether or not anti-leprosy drugs prevent AD, many researches began to study its effect, both *in vitro* and *in vivo* [94, 95].
Tomiyama *et al*. [94], demonstrated that rifampicin inhibited aggregation and fibril formation of synthetic Aβ1–40 and as well as having the strongest activity against the accumulation and toxicity of intracellular Aβ oligomers in PC12 cultured cells, but not dapsone. This protective effect may be achieved by scavenging ROS as well as by inhibiting Aβ aggregation and fibril formation in a dose dependent manner and/or the oligomer–cell membrane interaction. Chemical structure of rifampicin has a naphthohydroquinone ring [96] which is involved in its ROS scavenger role by oxidizing to quinone. Also, it may bind to Aβ by hydrophobic interaction between its lipophilic ansa chain and the hydrophobic region of the peptide blocking association between peptide molecules (Fig. **3**). On the contrary, Mindermann *et al*. [97], also showed that the ansa chain of rifampicin was not essential for the Aβ aggregation inhibitory activities while its lipophilicity contributed significantly to the transportation of the molecule into the brain *in vivo*.
Rifampicin and its analogues, p-benzoquinone and hydroquinone, inhibited the toxicity of preformed aggregates of Aβ polypeptide by binding to peptide fibrils, recognizing a certain conformation, preventing amyloid-cell interaction. Therefore, this antibiotic may mediate the conversion of plaque Aβ from toxic oligomers to non-toxic fibrils *via* monomers [98 - 101]. Similar studies have evaluated the anti-amyloid effect of rifampicin on Aβ aggregation and related toxicity and have revealed that the inhibitory effect was induced more by their binding to peptide fibrils than by their intracellular antioxidant action [102, 103]. Furthermore, rifampicin may promote the efflux of amyloidogenic proteins from

the brain into the periphery. Rifampicin has been shown, both *in vitro* and *in vivo* to facilitate Aβ clearance by upregulating the expression of LRP1 and P-gp at the BBB and such clearance may be more efficient for protein monomers than for oligomers [9, 10, 104]. In this line, recently Kaur *et al*. revealed that rifampicin significantly improved memory dysfunction and locomotor impairment in a rat dementia model. They confirmed the activity known to date of rifampicin against Aβ accumulation and neurotoxicity. They found that this antibiotic significantly reduced the oxidative stress, neutrophilic infiltration and amyloid deposition [105].

Moreover, this antibiotic has anti-inflammatory properties by inhibiting microglial activation. It has been described that rifampicin inhibits the LPS-stimulated expression of TLR-2 and TLR-4 and improves neural survival against inflammation and also can inhibit the production of proinflammatory factors through downregulation of NF-κB and MAPKs pathway [106 - 108]. Finally, this antibiotic exerts antiapoptotic properties that provide neuroprotection [108, 109].

Umeda *et al*., [95], showed that, when orally administered to different mouse models of AD and tauopathy, rifampicin reduces the accumulation of Aβ oligomers inhibiting aggregation and promoting secretion from cells. It also reduced tau protein hyperphosphorylation, synapse loss, microglial activation in a dose-dependent manner, inhibited cytochrome c release from the mitochondria and caspase 3 activation in the hippocampus and improved the memory of the mice, examined using the Morris water maze. Additionally, these authors suggest that this antibiotic restores autophagy-lysosomal function by preventing abnormal protein accumulation beyond the capacity of the protein-degrading system. Rifampicin significantly reduced the levels of p62 in transgenic mice without affecting LC3 conversion.

This results together with its pharmacokinetic properties make rifampicin a suitable medicine to treat neurodegenerative diseases that show extracellular and intracellular protein aggregates in the CNS, such is the case with AD and it has been proposed as being a promising medicine for the prevention of AD and other neurodegenerative diseases [87, 102, 103, 110].

On the other hand, Endoh *et al*. [93], did not find any effect of dapsone or rifampicin on Aβ neurotoxicity using mouse neuronal cultures. Also, an *in vivo* study showed that dapsone does not seem to decrease the production of Aβ in mice [111]. Moreover, a 1-year, randomized, double-blind, placebo-controlled study carried out with dapsone in people with AD was unsuccessful. Dapsone (100 mg/day) and placebo were administered orally, once daily for 52 weeks in 201 people with mild-to-moderate AD. At the end of treatment, there were no significant differences between dapsone and the placebo on either the cognitive or other measures of efficacy. There is, however, a great deal of further analysis yet to be done and specific attention will be paid to the analysis of subgroups of patients [112 - 115].

In contrast to the numerous pre-clinical studies, only a few clinical studies have analyzed the neuroprotective effects of rifampicin in people with AD. None of the trials used rifampicin alone but combined with doxycycline.

Doxycycline, part of the tetracycline antibiotic group, as well as rifampicin, is able to cross the BBB. Doxycycline is used to treat infections caused by bacteria with a broad therapeutic spectrum and there is little evidence of serious adverse events. It is avoided during pregnancy because other tetracyclines have been associated with transient suppression of bone growth and with staining of

developing teeth. Doxycycline may also be used for the treatment of malaria, sexually transmitted infection, and to treat or prevent Lyme disease [116].
Related to AD, *in vitro*, doxycycline, disassembles Aβ fibrils [117] and suppresses mutant tau protein production in transgenic mice [118]. It also has an anti-inflammatory effect [119, 120]. In a *Drosophila* model of Aβ toxicity, doxycycline prevents Aβ fibrillation by generating non-toxic structures. These results were also confirmed *in vitro* in a neuroblastoma cell line [121].
Thus, Loeb *et al*. [122], developed a pilot study where oral daily doses of doxycycline 200 mg and rifampin 300 mg for 3 months. This showed to have a therapeutic role in people with mild to moderate AD, improving their cognitive function measured with the Standardized Alzheimer Disease Assessment Scale-Cognitive subscale (SADAScog score). This treatment also reduced blood Creactive protein (CRP) levels supporting the anti-inflammatory role of these antibiotics.
However, a later clinical trial (ISRCTN15039674) called the DARAD study, designed to confirm or refute these promising pilot results, did not show any beneficial effect on cognition or function in people with AD as a result of taking rifampicin (300mg) or doxycycline (100mg) alone or in combination after twelve months of treatment [123].
The same group registered in 2007 the study NCT00439166. The goal of this multi-centered, randomized, controlled trial, was to determine if the biomarkers Aβ1-40 and Aβ1-42, Phospho-tau and total-tau protein, matrix metalloproteinases (MMP-2, MMP-9), pro-inflammatory cytokines (IL-1β, TNF-α) and antiinflammatory cytokines (IL-4 and IL-10) present in the cerebrospinal fluid (CSF) of people with AD were affected by treatment with doxycycline and rifampicin at the start and one year after treatment (100 participants). The last register of this phase III trial was in March of 2018. Although it is completed, no result has been published yet. Likewise, the same team registered a pilot study (Phase III) in 2008 with 21 participants (NCT00715858) whose objective was to compare inflammatory biomarkers in blood and CSF in people with AD and age-matched controls and theirresponse to the antibiotics doxycycline and rifampin. The results of this preliminary analysis would be used in defining the direction of further research. On the other hand, the observational clinical trial NCT00692588, was a prospective study started in 2008, aimed at analyzing the changes in brain structure and function using MRI scans in patients who participated in the DARAD study comparing changes pre *versus* post treatment and normal controls *versus* people with AD, in order to provide more definitive information about the promising use of antibiotics as a treatment. Lastly, in 2010, a clinical trial in phase I (NCT01002079) named the Drug-Drug Interaction Study with Rifampin, was started. The purpose of this study was to determine if the concomitant administration of rifampicin with BMS-708163 in healthy subjects will affect the pharmacokinetics of BMS-708163, a potent, selective and orally bioavailable γ-Secretase Inhibitor [124] and to assess safety and tolerability of co-administration BMS-708163 and rifampicin. In any case the results have yet to be published.
Recently, Izuka *et al*. [125], examined whether rifampicin has a preventive effect on humans. These authors retrospectively reviewed 18F-FDG-PET findings of elderly patients with mycobacterium infection treated with rifampicin. Forty nondemented elderly patients treated with rifampicin for mycobacterium infections who showed AD-type hypometabolism were enrolled. That way, they could evaluate the effect of rifampicin prior to the onset of dementia by using FDGPET,

as rifampicin was administered not for the clinical trial but for the treatment of mycobacterium infections. Having AD-type hypometabolism indicated that the patients were in a state of readiness to decline and this timing seemed optimal to start preventive therapy. Clinical trials of therapies that target Aβ in people with AD have revealed that initiating therapy after the onset of clinical symptoms has little effect on cognitive function. The results showed that the preventive effect of rifampicin depended on the dose and the treatment duration, namely at least 450 mg daily for one year to produce the desired effect.

On the whole, despite the strong evidence for the beneficial effects of rifampicin in cells and animal models of AD (Fig. **4**), there is not agreement about its role in humans. Hence, further studies are necessary to confirm the neuroprotective effect of rifampicin alone or in combination with other antibiotics such as doxycycline and to evaluate their clinical relevance.

Rapamycin

Vezima *et al*. in 1975 obtained *Streptomyces hygroscopicus*, bacteria from the soil of Rapa Nui (Eastern Island) from which rapamycin was isolated [126]. This antibiotic exerted no effect on any bacterial species and was described as an antifungal activity, but the use for this purpose in humans was quickly discarded mainly due to its immunosuppresive effects, as these adverse effects are totally linked to the development of some tumors [127]. However, it was in 1999 when the FDA approved its clinical use for kidney transplant rejection therapy and it was precisely due to its properties as an immunosuppressor [128]. It inhibits the IL-2 induced signal [129] and cell cycle progression in T lymphocytes [130]. On the other hand, surprising results were obtained using this compound on different animal species. It increases life expectancy in nematodes (*Caenorhabditis elegans*), insects (*Drosophila melanogaster)* and laboratory rodents [131 - 133]. In mice, rapamycin exerts a positive effect in lifespan and healthy aging, especially in females, even when the treatment begins in older subjects (19 months of age) [133]. Additionally, many researchers have focused their studies on the effects of this compound and its analogs in different pathologies including coronary stenosis [134], polycystic kidney disease [135] and different cancer types such as the glioblastoma multiform [136] finding, which in general shows a positive effect. One of the pathologies in which more research groups throughout the world are interested in, is AD. This idea is based on the inhibitory effect of rapamycin on mTOR pathway and its connection to AD. This kinase exerts a fundamental role in protein synthesis and proteolysis control which depends on its capacity to phosphorylate, more than 800 different proteins, either directly or indirectly. The overactivation of this pathway has been involved in some of the pathogenic changes related to AD development including neuroinflammation, the formation of neurofibrillary tangles, amyloid beta plaque and the inhibition of autophagosome. The mTOR pathway and the mechanisms through which mTOR participates in the pathophysiology of this disease are explained in more detailed later [137 - 139]. These data are crucial to understanding the importance of rapamycin as an eventual therapeutic drug for Alzheimer´s.

In 1993, the protein complex through rapamycin exerted its toxicity in yeast; Target of Rapamycin (TOR) was discovered [140, 141]. It was named mTOR in mammals due to its homology to the yeast TOR/DRR genes [142] and it is downstream activated by different factors such as hormones, growth factors, nutrients, stress, amino acids and it exerts a central role in cellular metabolism, cell motility, growth and survival. The interest in studying mTOR is related to its

role in the development of several brain neurodegenerative processes including Huntington´s disease, Parkinson´s disease, Down syndrome and AD [143, 144]. In the latter case, many studies have demonstrated an mTOR pathway dysregulation in brains of people with AD and in animal models [145 - 147] and these findings suggest that rapamycin or its analogs could be a valid drug for AD treatment. This hypothesis is based on the knowledge about the mTOR structure and function that is reviewed below.

mTOR controls many basic cell functions including mRNA transcription, protein synthesis, proteolysis, lipid biogenesis, mitochondrial metabolism, autophagy, cytoskeleton assembly, cell motility and cell growth [148 - 151]. mTOR constitutes two possible different structural and functional complexes; mTORC1 and mTORC2, which exert different roles in cell activities possibly related to a control of cell function in a precise temporal and spatial manner [152]. Starting with mTORC1, it is composed of mTOR, the regulatory protein associated with mTOR (RAPTOR), the non-core components PRAS40, the DEP domaincontaining mTOR interacting protein (Deptor), Tti/Tel2 complex and the mammalian lethal with SEC 13 protein 8 (mLST8) [153]. Once the complex is activated, Raptor binds to factor 4E-binding protein (4EBP1) and ribosomal S6 protein kinase p70 S6 kinase (p70S6K), inducing phosphorylation of 4EBP1 enhancing p70S6K kinase activity. Then, ribosomal biosynthesis, by activating RNA polymerase III dependent transcription and protein synthesis capacity are up-regulated. It also controls energy homeostasis regulating the mitochondrial oxidative activity and biosynthesis of mitochondria and these effects are related to cell cycle progression, size increase and survival. Then, it acts as a regulator of cell growth, exerting different effects on protein homeostasis, nucleotide biosynthesis, lipogenesis, glycogenesis and autophagy [154 - 164]. It is important to highlight the role that mTOR plays in autophagy and protein homeostasis, given its involvement in the development of AD. In general, when a cell has enough nutrients, mTORC1 inhibits autophagy by phosphorylating different kinases including ULK, which are related to the formation of the preautophagosome in mammals through mechanisms not completely understood [66, 67].

Finally, it is necessary to mention that part of the activity of mTORC1 depends on PI3K/Akt activation, which in turn activates mTORC1 and participates in the effect of 4EBP1 and p70S6K being that these pathways are deeply related to each other [162, 165]. But, what is the relationship between mTORC1 and rapamycin? Rapamycin inhibits mTORC1 and this effect depends on the intracellular receptor FKBP12 which interacts to rapamycin in turn inhibiting mTORC1 [166]. Then, mTORC1 activity is regulated by rapamycin, but also by hormones such as insulin, growth factors as IGF-I, amino acids, mechanical stimuli and oxidative stress [167].

mTORC2 exerts important actions through F-actin, paxilin, RhoA, essential for actin cytoskeleton function. Moreover, this complex phosphorylates Akt/PKB protein kinase on Ser473, which is related to survival and metabolism [152]. Akt is also phosphorylated on Thr473 by mTORC2 which facilitates the phosphorylation of Akt by PDK1. Then Akt is fully activated [168]). mTORC2 also phosphorylates IGF-1 receptor and insulin receptor, through its tyrosine protein kinase activity [169]. The mTORC2 complex consists of rapamycininsensitive companion of mTOR (RICTOR), mLSTR8, Deptor, Tti/Tel2 complex, mammalian stress-activated MAP kinase-interacting protein 1 and mTOR. It was

previously considered that only mTORC1 was inhibited by rapamycin [170]. However, Sarbassov *et al*. demonstrated that mTORC2 activity is also reduced when cells are treated long term *in vitro* [171] (Fig. **5**).

In the last decade, the relationship between AD development and mTOR complex activity has been investigated in many laboratories around the world, and some of the data obtained are described below. A common early change that occurs in neurons from amnestic mild cognitive impairment and late-stage AD subjects is the increase of mTOR phosphorylation [172]. The hyperphosphorylated residues are Ser2448 and Ser2481, in the temporal lobe of AD human brains, respecting their controls [173]. mTOR phosphorylation itself is altered, but also many other kinases related to this pathway including phosphoinositide 3 kinase, Akt and p70S6K [174, 175]. An *et al*., studied the temporal lobe from people with AD and demonstrated a positive correlation between neurofibrillary neuron alterations to p70S6K phosphorylation (at Thr421/Ser424) [165]. Thus, the overactivation of mTOR-p70S6K is related to the accumulation of abnormally hyperphosphorylated tau protein and decrease in dephosphorylation [165]. The eIF4E phosphorylated levels are also elevated, especially in late stages, and it is again correlated to hyperphosphorylated tau protein [174]. These data suggest that the changes in eIF4E and p70S6K may be related to neurofibrillary accumulation, being that proteolytic system is unable to compensate for the excess of these proteins. Furthermore, as it has been previously described, the autophagy is one of the impaired mechanisms in AD and some of the factors related to the biosynthesis of autophagosomes has been previously described in this report. Thus, mTOR overactivation seems to be implicated in this process through the inhibition of kinases as ULK [66, 67] and LC3 [68].

Aβ and hyperphosphorylated tau protein produce aggregates which are accumulated in AD leading to neuron death in AD [1, 2]. mTOR inhibits the transcription of genes related to autophagosome induction [68, 176]. Then, mTOR ameliorates Aβ clearance in AD brain [146, 177, 178], Moreover, when mTOR is inhibited by rapamycin or its analogs, autophagy genes are transcribed [179, 180], autophagosome formation is increased and consequently, Aβ accumulations reduced. This has been proved both *in vitro* using 7PA2 cells, as *in vivo* using AD mice models [138]. Finally, it has been demonstrated the role of mTOR in the clearance of other pathological aggregates such huntingtin in Huntington disease mice model [181].

It is well known that learning and memory are affected by AD and some researchers have focused their studies on the relationship between mTOR and memory impairment of this disease. Thus, Lang *et al*., demonstrated that four weeks of Everolimus (Rapamycin) treatment improved logical memory, mood and life quality in heart transplant patients [182]. On the other hand, Yates *et al*., using blood lymphocytes samples obtained from people with AD, demonstrated a positive correlation between mTOR pathway dysfunction and cognitive impairment [183]. They proposed that this peripheral modification in the phosphorylating levels of mTOR in these cells could be used as a predictive indicator of AD development. These changes depend on the important role of mTOR in synaptic plasticity which is important for learning and memory. One of the early changes in AD is the reduction in the expression of some of the proteins such as SV2, SNAP-25, synaptophysin and synaptotagmin, which are related to synaptic function and the release of neurotransmitters [184 - 186]. mTOR is related to this effect because rapamycin is able to revert the effect exerted by Aβ,

increasing some of these proteins such as SV2 expression [187]. Rapamycin is also able to increase the frequency of miniature excitatory postsynaptic currents after Aβ induced synaptotoxicity in primary hippocampal cultures obtained from mice [187]. Although all these studies demonstrated a negative effect of mTOR on synaptic plasticity, Tischmeyer *et al*. proposed that long term memory consolidation depended on mTOR activation [188]. This is an apparent important discrepancy, but it could be due to differences in the magnitude of the mTOR pathway phosphorylation in both cases.

One important question that should be asked is: What is the mechanism responsible for the overactivation of mTOR in AD? The plausible candidate is Aβ and this hypothesis was elegantly demonstrated by Caccamo *et al*., using a genetic mice model of AD in which mTOR is not overactivated when Aβ accumulation is prevented by the use of the Aβ antibody (6E10) [189]. On the other hand, Aβ directly injected into wild type mice hippocampus causes mTOR overactivation and this effect is mediated by the proline-rich Akt substrate 40 (PRA40). There is much evidence suggesting that the role of Aβ in mTOR activation. Hence, both Akt and p85 subunit of PI3K are overphosphorylated at Ser473 and Tyr508 in people with AD [172] and the increase of Aβ1-42 overactivates the PI3K/Akt/mTOR axis which in turn affects proteostasis [138]. Furthermore, embryonic cortical neurons from mice treated with Aβ oligomers presented neurite and cell cycle alterations and these effects occurred through PI3K, Akt/mTOR activation [190]. There is an alternative way which increases the activation of these pathways and depends on the insulin receptor. Therefore, it is well known that AD is associated to alterations in the brain´s glucose uptake capacity. Some of the studies are focused on the study of the role of the insulin receptor sustrate-1/2 (IRS1/2) because in AD brains, Aβ oligomers increase its phosphorylation at Ser636, 312, 616. These are inhibitory residues and their phosphorylation also mediates the overactivation of Akt and mTOR [191]. However, it is important to highlight that there is a report which has demonstrated a negative effect of Aβ on mTOR signaling. The differences between this and the experiments mentioned above may be related to the dose of Aβ used, being cytotoxic in the latter case [192].

In conclusion, overwhelming results from *in vivo* and *in vitro* experiments demonstrate the important role of mTOR in AD development and its inhibition by rapamycin or its analogs seems to be a potentially good therapeutic strategy. It exerts a positive effect on autophagosome formation, Aβ accumulation, tau protein hyperphosphorylation and cognitive status. However, to our knowledge, no clinical assays have been performed with these drugs for the prevention or treatment of AD.

Minocycline

Minocycline is a semisynthetic second-generation tetracycline, used clinically as a broad-spectrum antibiotic, that can easily cross BBB because it is a highly lipophilic molecule. Normally minocycline possesses great popularity as an antiacne treatment and in rheumatoid arthritis therapy [83]. In comparison to tetracycline structure, minocycline presents a diethyl amino group to the ring D and its lack of functional groups in the ring C, which has been related to its nonantibiotic properties. Its bacteriostatic activity is based on binding the bacterial 30S ribosomal subunit, inhibiting protein synthesis [193]. These chemical modifications enhance tissue absorption of minocycline into the cerebrospinal fluid and CNS and give a longer half-life compared to the original tetracyclines

[194, 195] (Fig. **6**).

There is numerous evidence showing that minocycline exerts neuroprotective effects on experimental models of different neurodegenerative diseases with an inflammatory base such as Huntington´s disease [196], Parkinson´s disease [197], amyotrophic lateral sclerosis [198] and AD, performing different action mechanisms. Nonetheless, we should not forget that a suitable minocycline doses must always be used for carrying out our research. It has been shown that a prolonged minocycline treatment decreased the survival of motor neurons and glial function in the organotypic rat spinal cord cultures [199]. In this same way, a study has reported that high doses of minocycline may induce delayed activation of microglia in aged rats and thus cannot prevent cognitive decline [200]. In humans, intravenous minocycline administration is safe and well tolerated for up to a doses of 10 mg/kg and has been shown to be neuroprotective in experimental trials [201]. Therefore, a concentration lower than 100 M *in vitro* studies and a dose up to 10 mg/kg of body weight *in vivo* studies should be used to analyse the neuroprotective properties of minocycline. However, we should not forget that the long-term minocycline treatment can trigger some rare adverse effects such as hyperpigmentation of the lower extremities, vomit, diarrhea, anorexia, thrombocytopenia and hepatic insufficiency and renal failure in the elderly population [202].

The minocycline anti-inflammatory properties were the first non-antibiotic activity reported due to its capacity to reduce microglial activation and levels of inflammatory mediators. Yrjänheikki´s group was the first to attribute antiinflammatory properties to minocycline in an ischemia cerebral rat model [203].

AD-associated neuroinflammation is a vicious circle, since Aβ induces microglial production of pro-inflammatory cytokines which favour the Aβ formation and aggregation at the same time [204]. Several studies have found that minocycline can improve cognitive decline by suppressing microglial production of proinflammatory cytokines, IL-1β, IL-6, and TNF-α, *in vitro* as well as *in vivo* in models of AD [205, 206]. Moreover, the minocycline-induced decrease in NF-kβ gene expression reduces cytokines production in rhesus monkey brain astrocytes and microglia, because NF-kβ is a cellular stimulator of pro-inflammatory cytokines synthesis [207].

Different *in vitro* studies demonstrated that minocycline blocked LPS-stimulated inflammatory cytokine secretion in the BV2 microglia-derived cell line and on microglia isolated from the brains of mice [208 - 210]. Nevertheless, an *in vitro* and *in vivo* study by Vay *et al*. demonstrated that minocycline not only reduced the activity of proinflammatory cytokines but mitigated the cytokine-induced astrocytic differentiation and microglia activation and recovered the neurogenic and oligodendrogliogenic potential of neural stem cells as well, counteracting the devastating effect of neuroinflammation [211]. Subsequently, many *in vivo* studies have followed corroborating the anti-inflammatory role of minocycline in AD. In 2004, using an experimental model of AD in mice, Hunter described, for the first time, that minocycline reduces cholinergic fibre loss in the hippocampus, ameliorates microglial and astrocytic activation induced by toxins and attenuates pro-inflammatory cytokines secretion as well as cognitive impairment [212]. Also, Biscaro *et al*., demonstrated that the minocycline reduced microglial cell activation, normalized IL-6 and increased levels of anti-inflammatory IL-10, which is a known inhibitor of TNF-α, improving cognitive deficit in a doubly transgenic (APP and mutant human PS1) mouse model of AD [213]. A recent

article has reported that minocycline reduces inflammatory parameters in different brain areas and serum as well as reverses the cognitive decline induced by the administration of Aβ1-42 in mice [214]. Additionally, Clemens *et al*., have suggested that anti-inflammatory effects of minocycline appears to predominantly be mediated by retinoid signalling, being that the postulated first mode of action for minocycline in human microglial-like cells. They observed that minocycline treatment blocked the retinoic acid degradation in adose-dependent manner, enhancing retinoid acid levels and reducing pro-inflammatory cytokines in microglial cells [215].

Therefore, all the findings obtained so far encourage the scientific community to fully trusts in minocycline´s therapeutic capacities which could mitigate the AD associated neuroinflammation.

Minocycline is also considered an anti-amyloidogenic agent because it reduces the Aβ production and potentiates the Aβ clearance. Additionally, a recent Food and Drug Administration (FDA) approved study demonstrates that minocycline is, among other drugs tested, the most effective for preventing Aβ1-42-induced toxicity and oligomerization in PC12 neurons [216]. Several studies have reported that minocycline administration affects Aβ deposits in APP transgenic mice [217]. In addition, this antibiotic inhibits the formation of Aβ fibrils in the post-mortem brains of people with AD [218]. Furthermore, it has been shown that minocycline decreases Aβ production through the inhibition of β-secretase (BACE1), the main enzyme responsible for the amyloidogenic processing of APP [219]. Regarding Aβ clearance, minocycline increased neprilysin expression, an Aβ-degrading enzyme, in the brains of Aβ-treated rats, preventing the appearance of senile plaques [220]. In this same vein, microglial phagocytic activity plays an important role in Aβ degradation, being reduced during the aging process. Initial studies described that minocycline does not modify the phagocytic capacity of microglial cells [221]. Conversely, a study has demonstrated that minocycline enhances Aβ fibrils phagocytosis in primary microglial cells [222]. Moreover, recent studies have found that autophagy, an important regulator system of protein cellular homeostasis necessary for a health brain, plays a dual role in the metabolism and secretion of Aβ in AD disease. This process is very impaired in AD as shown by the presence of autophagosomes surrounding the senile plaques nucleus within the AD brain [223]. Nilsson *et al*., observed that autophagy-deficient APP transgenic mouse had a reduced extracellular plaque load and Aβ levels in the brain [224]. Likewise, it has been shown that minocycline is able to induce autophagic cell death in glioma cells, inhibiting the growth of tumour [225] However, the effect of minocycline on AD-associated autophagy has not been studied so far. Previously obtained findings in other pathologies, suggest that this antibiotic could also be a good candidate for boosting autophagy in AD [226]. Hence, further study is necessary in order to describe the effect of minocycline on AD associated autophagy. Also, a well-functioning autophagy warrants a healthy brain because neurons with complex axonal and dendritic structures are dependent on intense transport and efficient protein turnover in order to allow for a suitable synapsis and neuroplasticity [227]. In AD, it is well known that both neuronal function and intercellular communication is completely impaired in humans and murine models of this disease [228]. However, various studies showed that minocycline greatly improves learning and memory by augmenting neuroplasticity and synaptogenesis in the hippocampus of aged mice. The brain derived neurotrophic factor (BDNF) levels, a regulator factor of synapsis

plasticity and memory, are increased by minocycline, as well as the reduction of synapse-associated proteins in hippocampus of aged mice [229]. Furthermore, it has been demonstrated that minocycline promotes neurite growth in PC12 cells after oxygen-glucose deprivation, which supports the beneficial effect of minocycline on the neuroplasticity even more [230]. Along the same line, several studies demonstrated that the Akt- mediated cellular survival pathway is aberrant and is related to cognitive alterations in AD [231]. However, minocycline is able to potentiate cell survival as well as cell damage prevention by PI3K/Akt signalling pathway [232, 233]. Again, all investigations seem to lead to the beneficial effect of minocycline in this devastating neurodegenerative disease.
Aβ accumulation also provokes harmful effects on some neurotransmitters involved in learning and memory such as somatostatin, dopamine and glutamate. The expression levels of somatostatin and dopamine are decreased [234, 235] as well as the glutamate transporter-1 from the human temporal cortex in brains with AD [236]. Minocycline prevents Aβ-induced reduction of somatostatin [220] and protects the somatostatin receptor-effector system from Aβ-induced alterations in an experimental model of AD [237]. Regarding dopamine, several studies have shown that minocycline prevents the dopaminergic neurodegeneration typical of Parkinson´s disease and other closely related conditions of memory loss and mood in people with AD [238 - 240]. Furthermore, the minocycline heightens the spinal excitatory glutamatergic transmission in lamina II rat neurons [241]. Therefore, the impaired neurotransmission systems typical of AD might be improved with minocycline treatment.
Considering the neurofibrillary tangles of hyperphosphorylated tau protein, minocycline decreases the production of abnormal tau protein species in *in vitro* and *in vivo* animal models of AD [242]. At the same time, studies carried out using a transgenic mouse model for AD have shown that minocycline restores hippocampus, cortex and amygdala-dependent learning and memory deficits [243] adding another cognitive effect to this tetracycline.
In summary, all pre-clinical findings indicate that minocycline exerts a great range of neuroprotective effects on AD. However, there are not enough clinical trials to test these properties in people with AD. Currently, there is only one completed clinical trial (NCT01463384), but the results are not conclusive because the number of patients studied is not very high. Also, the National Institute of Health Research (NIHR) is carrying out a clinical trial for AD to evaluate the effects of two years of minocycline treatment on the deterioration of the mental processes in people with early AD (ISRCTN16105064). In the last update (18 August), they reported that all findings have been collected and that they are currently analysing these data.
Therefore, proper designed clinical trials are required to extrapolate minocycline neuroprotective properties from pre-clinical studies in humans. Likewise, more studies need to be carried out in order to describe the possible action mechanisms of minocycline.

Other Antibiotics

Macrolides

There are two main macrolide antibiotics which have been studied in the treatment of AD, azithromycin and erythromycin. On one hand, azithromycin, which acts as antibacterial inhibiting the 50S ribosome subunit during translation [244]. This semisynthetic aminoglycoside antibiotic has been used to treat different pathological stages in various AD models [245] because it is an excellent

inhibitor of APP 5´-untranslated region (5´-APP-UTR) of mRNA encoding the APP and it also intervenes at the overlapping signaling pathway to activate α and/or γ-secretase cleavage [246]. Therefore, azithromycin can modulate APP processing because it alters the cleavage of APP and it can also modify the processing of APP in human lens epithelial (B3) cells and in human neuroblastoma (SH-SY5Y) [247]. Recent advancements in drug development highlight this antibiotic as a new possibility in targeting the 5´-APP-UTR in AD. More than 1000 drugs related to this have been preapproved by the FDA, but only 17 have been found to be effective in inhibiting 5´-APP-UTR. However, it is important to mention some possible side effects of azithromycin, like nausea, abdominal pain, vomiting, flatulence, diarrhea and an increase of several liver enzymes [245].

On the other hand, erythromycin, which is an analog of azithromycin, was also investigated in similar studies using an AD mouse model. Both antibiotics markedly change the cleavage of the APP C-terminal fragment in SH-SY5Y cells [248]. Like azithromycin, it alters APP processing resulting in a diminution of cerebral levels of Aβ(1-42) without having any effect on Aβ(1-40) levels. Erythromycin has been postulated as a possible neuroprotective agent because it induces the expression of a 7-kDa APP C-terminal fragment, which may increase the expression of different neuroprotective target genes [249]. This antibiotic also reduced the amount of C99 and C83 C-terminal fragments (both are substrates of g and a-secretase, respectively, for the generation of Aβ) in the brain cortex of the transgenic mouse model for AD TgCrND8 relative to controls. A postulated explanation for erythromycin action could be that this antibiotic would activate the γ-secretase complex to switch APP cleavage specificity towards the Aβ(1-42) cleavage site way the Aβ(1-40) cleavage site [248].

To summarize, all these pre-clinical studies indicate that the main role of these two macrolide antibiotics in the treatment of AD is due to the fact that they can modify the APP processing.

Clioquinol

Clioquinol (5-chloro-7-iodo-8-hydroxyquinolinol) is a hydroxyquinoline with antifungal and antiprotozoal properties which is prescribed in some topical preparations in order to treat different skin infections [249] and is described as neurotoxic when it is used chronically at high doses [245]. Clioquinol was extensively used in the past as an anti-infective, especially for diarrhea. In 1970, it was withdrawn from oral use after being associated with subacute myelo-optic neuropathy (SMON), a Japanese neurotoxic epidemic [250]. This syndrome is characterized by upper and lower motor neuron lower limb signs and subacute visual changes (optic neuritis). The physical signs of SMON are similar to those of subacute combined degeneration of the cord secondary to vitamin B12 deficiency. Therefore, for people treated with clioquinol, SMON could be avoided by the administration of vitamin B12 [251].

This antibiotic is a hydrophobic metal protein attenuating compound which can cross the BBB. It has a greater affinity for Cu^{2+} and Zn^{2+}, two metal ions critically involved in amyloid-β aggregation and toxicity [252], than for Ca^{2+} and Mg^{2+} ions. In the ´amyloid cascade´, Clioquinol might act by either preventing Aβ deposition into amyloid plaques or by promoting Aβ clearance through the mobilization of Aβ from existing deposits [251]. Specifically, Clioquinol can prevent the formation of amyloid plaques in transgenic AD mice and dissolve Aβ from postmortem tissue of people with AD [251, 253]. Therefore, clioquinol may act

by disaggregating collections of Aβ and Cu, `dissolving´ accumulations of Aβ [254]. However, an extended clioquinol treatment in a different mouse model led to a reduced survival [255]. Some clinical trials of clioquinol suggested a significant slowing down of the cognitive decline in the most severely affected subgroup of people with AD, with a parallel reduction in plasma Aβ1-42 levels. In addition, clioquinol was also reported to ameliorate disease pathology in a mouse model of Parkinson´s disease and Huntington´s disease [252].

Because of its action mode, different conflicting hypotheses have postulated the ability of clioquinol to interfere with brain metal metabolism. These hypotheses suggest that it might work as a chelating agent. Others studies propose that this antibiotic may act as a `copper carrier´, behaving as an ionophore that facilitates membrane crossing to complexed copper [256]. It was experimentally proved that an increase in intracellular copper levels raises metalloprotease activities which enhance Aβ clearance [256].

Moreover, a series of selenium-containing clioquinol derivatives were designed, synthesized and evaluated as multifunctional anti-AD agents. *in vitro* examination showed that several target compounds exhibited activities such as the inhibition of metal-induced Aβ aggregation, antioxidative properties, hydrogen peroxide scavenging and the prevention of copper redox cycling. A parallel artificial membrane permeation assay indicated that selenium-containing clioquinol derivatives possessed significant BBB permeability [257].

In TgCRND8, a transgenic AD mouse, this hydroxyquinoline reverses to a large extent, the working memory problems that are characteristic of this mouse model, reducing amyloid plaques in the cortex and hippocampus region of the brain and attenuating astrogliosis. Furthermore, significant effects on the absolute and relative brain concentrations of the three most important biometals (Cu^{2+}, Zn^{2+} and Fe^{2+}) were highlighted following this treatment with clioquinol as well as its distribution within the brain mirrored areas implicated in memory and learning [252, 258]. These observations led to a clinical trial using cloroquinol in which thirty-six subjects with AD (cognitive subscale score of the Alzheimer´s Disease Assessment Scale (ADAS-Cog) 20-45; Mini-Mental State Examination score 10-24) were included in this randomized double-bind phase II study. After 9 months, the effect of the treatment was significant in the more clinically severe group (ADAS-Cog ≥25) as a reduction in plasma Aβ(1-42) levels in the clioquinol group was observed and an increased in the pacebo group. While plasma zinc levels also increase in the clioquinol group, copper levels remained unchanged. Overall, Clioquinol was well tolerated by subjects at a maximum oral dose of 375 mg twice per day. Furthermore, the planned phase III trial of clioquinol was abandoned and this compound has been withdrawn from development [258].

It has been also demonstrated that a combination of the administration of cloroquinol and inoculation with Aβ1−42 vaccines in the transgenic mouse model APP/PS1 was effective in significantly reducing the deposits of amyloid in the brains of these animals. Furthermore, this study reports that systemic clioquinol induces myelopathies in the dorsal lateral geniculate nucleus, which was almost devoid of amyloid plaques and is the primary site of retinal efferent projections *via* the optic nerve. Inoculation with an Aβ1−42 vaccine was also found to result in a significant increase in plaque-independent astrocytic hyperplasia in the dorsal part of the lateral septal nucleus which was also devoid of plaques, reflecting potential brain inflammatory processes [259].

In conclusion, several clinical trials show Clioquinol´s capacity for preventing Aβ

deposition and active the mobilization of Aβ from existing deposits, therefore being an interesting candidate to utilize in the treatment of AD. However, more studies are needed in order to check if its behavior as a chelating agent could make this a potential drug for interfering with brain metal metabolism.

Amphotericin B

Amphotericin B is a widely used membrane-active antifungal drug which has several known toxicities including multiorgan failure and potential death that would like to limit its use as a treatment for AD [249]. It has also antiprotozoal, antiviral and indirect antimicrobial activity through immune stimulation. Most intriguing are the reports of the antiprion activity of Amphotericin B and its derivatives. In fact, they have been shown to be among the very few agents, which can slow the course of prion disease in animal models. It is currently unclear as to what the mechanism of action could be [260].

This polyene macrolide antibiotic prevents fibrillation in amyloid disease in AD, as it binds specifically to Aβ 25-35 fibrils [261]. Amphotericin B seems to have a complementary face for amyloid fibrils but not the native protein. Moreover, this medication interacts specifically with Congo Red, a very well-known fibrilbinding agent. In addition, in kinetic fibril formation studies, Amphotericin B was able to significantly kinetically delay the formation of Aβ 25-35 fibrils at pH 7.4 but not insulin fibrils at pH 2 [260]. However, the ability of Amphotericin B to affect the conformational changes of neurotoxic soluble oligomers of amyloid peptides, in particular Aβ(1-42) peptide, has not been reported thus far. Notably, this drug was also shown to modulate the aggregation process of prion protein, but no mechanistic details have been provided. In addition, Amphotericin B has no measurable impact neither on the secondary structure nor on the timedependent aggregation profile of the amyloid peptide [261].

In summary, some *in vitro* studies supporting that the importance of this antibiotic in the treatment of AD is based on the prevention of the formation of amyloid fibrils which Amphotericin B has.

Tetracycline

Tetracycline is a classical antibiotic used to treat different infections and it has been proposed for AD therapy due to its effects on the aggregation of Aβ protein, particularly its ability to interact *in vivo* with the Aβ oligomers and aggregates in different AD animal models [249]. Specifically, this drug led to the formation of colloidal particles that particularly sequester oligomers, preventing the progression of the amyloid cascade [262].

Furthermore, this antibiotic reduces the resistance of Aβ1-42 to trypsin digestion [117] and it induces an increase in disassembly of preformed fibrils in AD mouse models [249]. These effects were dose dependent and specific to tetracycline as opposed to antibiotics in general. It has been reported that the mechanism of action of this drug is based on the induced changes in the secondary structure of Aβ protein, from soluble form to β-sheet-rich structures corresponding to the presence of oligomers and fibrils, disassembling both Aβ oligomeric and fibrillar β-sheet assemblies, restoring their non-amyloidogenic structures [262, 263].

Moreover, tetracycline-treated transgenic *Caenorhabditis elegans*, a simplified invertebrate model of AD, showed lowered oxidative stress reducing superoxide production and protects from the onset of the paralysis phenotype [262].

In summary, owing to all these findings, tetracycline has been described in several animal models of AD as a possible drug used against this disease because of its capacity to disrupt the amyloid cascade, among other effects.

Finally, Fig. (**7**) recapitulates the principal effects of all these antibiotics.

CONCLUSION

Evidence seems to drive in the same direction, confirming that antibiotics could be promising candidates in the prevention and treatment of AD, exerting beneficial effects and are relatively inexpensive. It is important to emphasize that they can cross the BBB and all of them exert neuroprotective activities. Among them, it is important to highlight the anti-amyloidogenic role exerted by rifampicin, avoiding the Aβ aggregation. We mention again the effect of Rapamycin on autophagy activity which favours the clearance of Aβ and the antiinflammatory
activity of minocycline that reduces the Ab-induced microglial activation. Likewise, all these cited antibiotics and some others analysed in this chapter exert beneficial effects on many of the pathophysiological features of AD. Nevertheless, it is important to highlight that long-term treatment with ntibiotics could cause adverse effects includingantibiotic resistance. Therefore, these issues should be considered in current and future clinical trials in order to determine the therapeutic dose range which exerts neuroprotection. In this chapter, we have tried to emphasize the potential neuroprotector role of some antibiotics, mainly used against infections. Notwithstanding, it is still necessary to conduct further, properly executed, clinical trials further in order to confirm all the cited properties of these antibiotics in people with AD.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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