Neuroprotective antibiotics 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. Currently, around of 46 million ~~of~~ people suffer from AD worldwide, and these data will be duplicate in 20 years. 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 familiar and socio-sanitary problem. Therefore, it is essential to find therapeutic strategies which are addressed to prevent, delay the onset, slow the progression and /or improve the symptoms of AD. Nowadays, the research lines focus on finding and identifying new drugs for reaching these improvements. In this article we have focused on review thoroughly the neuroprotective role, in AD of the antibiotics rifampicin, rapamycin and minocycline, because they reach quickly the brain and are very cheap. Likewise, we have found evidences both *"in vitro"* and *"in vivo"* studies, even some clinical trials about it, but minority. In a general view, all the antibiotics reviewed exert neuroprotection, because they act as an anti-inflammatory and anti-amyloidogenic agents.

1. INTRODUCTION

Alzheimer´s disease (AD) is a multifactorial disease, which induces progressive memory loss and cognitive decline, exacerbated by neurotransmitter deficits. The "amyloid theory", which is based on the overexpression and aggregation of amyloid beta peptide (Aβ), is believed to be one of the main causes of its etiology. The presence of extracellular senile plaques containing Aβ and intracellular neurofibrillary tangles of hyperphosphorylated tau protein are neuropathologically characteristic in brain from AD patients [1]. The Aβ peptide is formed from amyloid protein precursor (APP) by sequential enzymatic processing, in which different β-secretase and γ-secretase are involved [2]. Moreover, Aβ aggregations are also 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 [3] which ultimately ends a in progressive neuronal loss predominantly by apoptosis [4]. Even the impaired phagocytic activity of microglia favours the Aβ deposition, exacerbating memory loss [5].

It is note to consider that AD is an irreversible neurodegenerative disorder and one of the main aging-dependent maladies of the 21st century. Currently, around of 46 million people suffer from AD worldwide, and these data will be duplicate in 20 years. The global demographic trend indicates that population aging is quickly increasing. The WHO estimates that, by 2040, the proportion of world population aged ≥65 is reached to 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 familiar and socio-sanitary problem. In 2015, direct medical costs, social cost and the cost of informal care added up a total of US$ 818 billion at global level. Therefore, it is essential to find therapeutic strategies which are addressed to prevent, delay the onset, slow the progression and /or improve the symptoms of AD. Nowadays, the research lines focus on finding and identifying new drugs for reaching these improvements.

Inside drugs there are a wide range of components with different nature and therapeutic purposes, such as antibiotic, antipsychotic, antihypertensive that exert assorted neuroprotective effects in AD. In the literature there many studies both pre-clinical and clinical that demonstrate that these candidates may interact with AD-associated pathophysiological mechanisms, inducing beneficial effects. Even recent studies have attributed neuroprotective properties to some foods as extra virgin olive oil

(hydroxytyrosol) [6], grapes (resveratrol) [7], fresh fish (omega 3 fatty acids) [8] and beverages as green tea [9] and coffee [10]. In this article, we review the known neuroprotective effects of some antibiotics on AD development, because these inexpensive and interesting candidates, are able to penetrate the blood-brain barrier and so reach the brain, target organ of this disorder [11, 12]. Currently the rifampicin, rapamycin and minocycline are the antibiotics more used both in preclinical and clinical studies, so in this review, we will bring up date the neuroprotective role of these antibiotics on the AD. Also we will update briefly the actions of other antibiotics less researched.

2. ANTIBITOTICS

2.1. RIFAMPICIN

Rifampicin is an antibiotic with a very broad spectrum of activity, used in the treatment of mycobacterium infections, including tuberculosis and leprosy. Several *"in vitro"* and *"in vivo"* studies have described the multifunctional properties of rifampicin and it is proposed as a promising medicine for the prevention of AD and other neurodegenerative diseases.

Tomiyama et al [13] demonstrated, in cultured cells, that rifampicin had the strongest activity against the accumulation and toxicity of intracellular Aβ oligomers. This protective effect may be achieved by scavenging ROS as well as by inhibiting Aβ oligomerization and/or the oligomer–membrane interaction. Rifampicin and its analogues, p-benzoquinone and hydroquinone, inhibited the toxicity of preformed aggregates of human islet amyloid polypeptide by binding to peptide fibrils, by recognizing a certain conformation, preventing amyloid-cell interaction. So that, this antibiotic, may mediate the conversion of plaque Aβ from toxic oligomers to non-toxic fibrils via monomers. [14-17].

Furthermore, rifampicin may promote the efflux of amyloidogenic proteins from the brain ~~in~~to the periphery by upregulating the expression of low density lipoprotein receptor-related protein-1 and P-glycoprotein (P-gp) at the blood–brain barrier and such clearance may be more efficient for protein monomers than for oligomers [18, 19].

Finally, this antibiotic has anti-inflammatory properties by inhibiting microglial activation and improves neural survival against inflammation [20].

All those mechanisms may synergistically work to protect neuron from toxic oligomers.

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 blood–brain barrier. These pharmacokinetic properties make this antibiotic a suitable medicine to treat neurodegenerative diseases that show extracellular and intracellular protein aggregates in the CNS as AD [21-23].

Umeda et al [24] showed that, rifampicin orally administered to different mouse models of AD and tauopathy, reduces the accumulation of Aβ oligomers as well as tau hyperphosphorylation, synapse loss, microglial activation in a dose-dependent manner, inhibits cytochrome c release from the mitochondria and caspase 3 activation in the hippocampus and improved the memory of the mice. Besides, these authors suggest that this antibiotic restores autophagy-lysosomal function by preventing abnormal protein accumulation beyond the capacity of the protein-degrading system.

In contrast to the numerous pre-clinical studies, only a few clinical studies have analyzed the neuroprotective effects of rifampicin in patients with AD.

An epidemiological study shown that, in Japan, a group of rifampicin-treated patients with leprosy had a significantly lower incidence of dementia compared with an untreated group [25]. Histological analyses indicated that elderly no-demented leprosy patients in Japan showed significantly lower levels of senile plaques in the brain than non-demented non-leprosy subjects [26, 27].

Loeb et al. [28] developed a pilot study where oral daily doses of doxycycline 200 mg and rifampicin 300 mg for 3 months have a therapeutic role in patients with mild to moderate AD, improving their cognitive function measured with the Standardized Alzheimer Disease Assessment Scale-Cognitive subscale (SADAScog score). However, a later clinical trial, designed to confirm or refute this promising pilot results did not show any beneficial effect on cognition or function of either rifampicin or doxycycline alone or in combination after twelve months of treatment in AD patients [29].

Recently, Izuka et al. [30] examined whether rifampicin has a preventive effects in humans. These authors retrospectively reviewed 18F-FDG-PET findings of elderly patients with *Mycobacterium* infection treated with rifampicin. Forty non-demented elderly patients treated with rifampicin for mycobacterium infections who showed AD-

type hypometabolism were enrolled. The results showed that the preventive effect of rifampicin depended on the dose and the treatment duration, and the effect needs at least 450 mg daily for 1 year.

On the whole, despite the strong evidence on the beneficial effects of rifampicin in cells and animal models of AD, there is not an agreement about its role in humans. So that, further studies are necessary to confirm the neuroprotective effect of rifampicin alone or in combination with other antibiotics as doxycycline and to evaluate their clinical relevance.

Recently, two clinical trials in phase 3 have already finished. First, the goal of the study NCT00439166, was to determine if biomarkers Aβ(1-40) and Aβ(1-42), P-tau and T-tau, matrix metalloproteinases (MMP-2, MMP-9), pro-inflammatory cytokines (IL-1beta, TNF-alpha), and anti-inflammatory cytokines (IL-4 and IL-10) present in the cerebrospinal fluid of people with AD were affected by treatment with doxycycline and rifampicin at the start and one year after treatment. Secondly, the clinical trial NCT00692588, a larger scale study, aimed to analyze the changes in brain structure and function using MRI scans in patients treated for AD with antibiotics in order to provide a more definitive information about the promising benefit of using antibiotics as a treatment. However, no results have been yet published.

2.2 RAPAMYCIN

Rapamycin, produced by *Streptomyces hygroscopicus*, was firstly described as a fungi growth inhibitor without any effect on bacteria [31]. However, it exerted immunosuppressive effects, so the eventual use in humans as an antifungal was early discarded. However, scientific attention was focused on its immunosuppressive effects and as a result of this research in 1999 the FDA approved its use to prevent organ transplant rejection [32]. Although this is a considerable application, it has been demonstrated that this compound increases lifespan and healthspan in species as different such *Caenorhabditis elengans*, *Drosophila melanogaster* or rodents [33-35]. The data obtained in mice supposed a great impact because the positive effect of rapamycin on lifespan was evidenced when the drug was administered in late life [35].

The binding activity of this compound to the serine/threonine kinase “mammal Target of Rapamycin” (mTOR) constitutes the basis of the molecular mechanism responsible of its

effects. Two mTOR complexes have been described, one of them is mTORC1 which exerts multiple actions including autophagy or cell growth and protein synthesis decrease [36], and all of them are inhibited by rapamycin. The effect of mTORC1 on protein translation is mainly exerted by controlling the activity of eukaryotic initiation factor 4E-binding protein (4EBP1) and ribosomal protein S6 kinase-1 (S6K1) [37].

The mTOR signaling upregulation has been related to the development of AD both in animal models and in humans [38]. The inhibition of mTORC1 by Rapamycin reduces inflammation but also the formation of Aβ plaques and neurofibrillary tangles [39-41]. mTOR and Aβ presente~~d~~ a complex relationship and when Aβ is used in physiological concentrations, mTOR is upregulated in different cell types including mouse neuroblastoma cells or Chinese hamster ovary [42, 40]. This effect has also been demonstrated when Aβ oligomers are administered in hippocampus of mice *in vivo*. Finally, it is important to highlight the negative effect of mTORC1 on autophagy and is due to a reduction in the Unc-51-like kinase1 (ULK1) phosphorylation. This kinase initiates the autophagosome formation [43] and rapamycin potentiates this mechanism blocking mTORC1.

Finally, many studies have described a positive effect of rapamycin or its analogs on cognition and behaviour, not only in some AD models in mice [39-41], but also in humans [44]. In this study, Lang et al described an improvement in cognition exerted by Everolimus in humans after four weeks of treatment [44]. However, the effect of rapamycin analogs on memory remains unsolved due to some data obtained by Tischmeyer et al in Mongolian gerbils, in which mTOR signalling pathway in cortex contributes to long term memory consolidation [45].
Therefore, mTOR inhibition by rapamycin or its derivatives are potential therapeutic drugs for the prevention or treatment of AD, although there is not yet any clinical trial about it.

2.3 MINOCYCLINE

Minocycline is a semisynthetic second-generation tetracycline which is used clinically as an antimicrobial agent, being active against a wide range of gram-positive and gram-negative bacteria. Numerous evidences have reported that minocycline exerts

non-antibiotic neuroprotective effects on different animal models of neurodegenerative diseases due to its anti-inflammatory activity, by reducing microglial activation and the cytokine expression levels. Likewise, minocycline treatment exerts anti-amyloidogenic activity and reduces deficits in learning and memory by improving the receptor–effector system from some neurotransmitters

AD- associated neuroinflammation involves a vicious circle, since Aβ induces microglial activation producing pro-inflammatory cytokines which favour the Aβ formation and aggregation at the same time [46]. However, Yrjänheikki et al., granted anti-inflammatory properties to minocycline in an ischemia cerebral rat model [47]. Later, different *in vitro* studies demonstrated that minocycline blocked LPS-stimulated inflammatory cytokine secretion in BV2 microglia-derived cell line and on microglia isolated from the brains mice [48-50]. In 2004, using an experimental model of AD in mice, Hunter described for the first time that minocycline reduces cholinergic fibre loss in hippocampus, ameliorates microglial and astrocytic activation induced by toxin and attenuates the pro-inflammatory cytokines secretion as well as cognitive impairment [51]. Nevertheless, many studies have followed corroborating the anti-inflammatory role of minocycline in AD. A recent article has reported that minocycline reduces inflammatory parameters in different brain areas and serum as well as reverses cognitive decline induced by the administration of Aβ (1-42) in mice [52].

Minocycline is also considered an anti-amiloidogenic agent. Several studies have reported that minocycline administration affects ~~to~~ Aβ deposits in APP transgenic mice [53]. In addition, this antibiotic inhibits Aβ fibrils formation in post-mortem brains from patients with AD [54]. Even it has been showed that minocycline decreases the Aβ production by inhibiting of β-secretase (BACE 1), main enzyme responsible for amyloidogenic processing of APP [55]. With respect to Aβ clearance, neprilysin expression, a Aβ-degrading enzyme, was increased by minocycline in brain from Aβ-treated rats, preventing appearance of the senile plaques [56]. In this same line, microglial phagocytic activity plays an important role in Aβ degradation, being reduced during aging. Initial studies described that minocycline does not modify to phagocytic capacity from microglial cells [57]. Conversely, a study has demonstrated that minocycline enhances Aβ fibrils phagocytosis in primary microglial cells [58]. Therefore, it is necessary to carry out more studies in order to clarify the effect of minocycline on Aβ-phagocytosis.

Aβ accumulation also provokes harmful effects on some neurotransmitters involved to learning and memory such as somatostatin and dopamine. The expression levels of both neurotransmitters are decreased in brain with AD [59, 60]. Minocycline prevents Aβ-induced reduction of somatostatin [56] and protects the somatostatin receptor-effector system from Aβ-induced alterations in an experimental model of AD [61]. With respect to dopamine several studies have been described that minocycline prevents dopaminergic neurodegeneration typical of Parkinson´s disease and closely related to memory loss and mood in patients with AD [62-64].

Considering neurofibrillary tangles of hyperphosphorylated tau protein, this tetracycline decreases production of abnormal tau species in *"in vitro"* and *"in vivo"* animal models of AD [65]. At the same time, studies carried out in transgenic mouse model for AD have shown that minocycline restores hippocampus, cortex and amygdala-dependent learning and memory deficits [66, 67], adding another cognitive effect to this tetracycline.

In summary, so far all pre-clinical findings indicate that minocycline exerts a great range of neuroprotective effects in AD. However, there is no clinical trial to test these properties in patients with AD currently, only there is one clinical trial registered (NCT01463384) but the results have not been communicated up to now.

3. OTHERS ANTIBIOTICS

Included at great antibiotics family, the macrolides are another antibiotic group with therapeutic properties on AD, such as azithromycin and erythromycin. Both modify the APP processing in mice models of this neuropathology, reducing Aβ production and so cognitive decline. Particularly azithromycin reduces cerebral levels of Aβ (1-42) [68] and erythromycin induces the expression of APP fragments which may increase activation of neuroprotective target genes. [69]. Also it has been demonstrated that, amphotericin may delay the formation of Aβ, preventing cognitive deficit [70].

4. GENERAL CONCLUSION

All evidences seem to point in the same direction, confirming that antibiotics could be sufficiently efficient candidates to prevent or treat AD because they can cross blood barrier brain exerting beneficial effects, and are cheap drugs. Moreover, all of them have similar neuroprotective properties, such as anti-inflammatory and anti-amyloidogenic ones. However, it is still necessary to increase properly-executed clinical trials in order to demonstrate the cited properties on these antibiotics in patients with AD.

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