Universidade de Lisboa

Faculdade de Farmácia

Tetracyclines beyond the antibiotic

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Mestrado Integrado em Ciências Farmacêuticas

2019

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Monografia de Mestrado Integrado em Ciências Farmacêuticas apresentada à Universidade de Lisboa através da Faculdade de Farmácia

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2019

ABSTRACT

The antibiotic effect of tetracyclines has been widely studied and demonstrated over the years. However, tetracyclines have been reborn at the time of recovering sensibility and incorporating new components and more active. The applications of tetracyclines outside the scope of the microbiology has been focused in the fact that tetracyclines exhibit anti-inflammatory and anti-apoptotic effects and affect multiple processes such as angiogenesis, proteolysis and bone metabolism.

Minocycline (MC), the most powerful tetracycline of that period, became one of the most widely used compound of tetracycline's family, and was the last tetracycline to be introduced into the market in the 20th century. MC was approved to use in 1971 after a long pathway of modifications of the analogs. MC showed so many potential results against many models of neurodegenerative diseases, such as the anti-inflammatory and the anti-apoptotic effects.

The fact of being known the side-effects profile of MC, being considered a safe drug and inexpensive which allows the long-term use, and the high penetration in the BBB, makes this compound the ideal to use in stroke treatment but also in multiple sclerosis, spinal cord injury, amyotrophic lateral sclerosis, Huntington's disease, Parkinson's disease, and Alzheimer's disease.

However, despite MC is considered the most effective tetracycline derivative regarding neurodegenerative diseases, there are still limitations to its potential: the dose administrated to avoid undesirable side-effects remains a crucial issue.

Key Words: Tetracyclines, minocycline, neurodegenerative diseases.

RESUMO

O efeito antibiótico das tetraciclinas tem sido amplamente estudado e demonstrado ao longo dos anos. No entanto, as tetraciclinas renasceram no momento de recuperar a sensibilidade e incorporar novos componentes alguns dos quais mais ativos. As aplicações das tetraciclinas fora do escopo da microbiologia têm sido focadas no fato das mesmas exibirem efeitos anti-inflamatórios e anti-apoptóticos que afetam múltiplos processos, tais como angiogênese, proteólise e metabolismo ósseo.

A minociclina (MC), que é a tetraciclina mais poderosa, tornou-se um dos compostos mais amplamente utilizados na família das tetraciclinas e foi a última das tetraciclinas a ser introduzida no mercado no século XX. A MC foi aprovada pela Food and Drugs Administration (FDA) para uso humano em 1971, após um longo caminho de modificações dos seus análogos. A MC apresentou vários resultados potenciais contra muitos modelos de doenças neurodegenerativas, como os efeitos anti-inflamatórios e anti-apoptóticos.

São conhecidos os efeitos colaterais da MC o que a torna um medicamento seguro e barato, permitindo o seu uso a longo prazo e a alta penetração na barreira hemato encefálica (BHE). Este fato torna o composto ideal para uso no tratamento de acidente vascular cerebral, mas também na esclerose múltipla, lesão medular, esclerose lateral amiotrófica, doença de Huntington, doença de Parkinson e na doença de Alzheimer.

No entanto, apesar da MC ser considerada o derivado das tetraciclinas mais eficaz em doenças neurodegenerativas, ainda existem limitações ao seu potencial: a dose administrada para evitar efeitos colaterais indesejáveis permanece como uma das questões cruciais.

Palavras-chave: Tetraciclinas, minociclinas, doenças neurodegenerativas.

ACKNOWLEDGEMENTS

Gostaria de expressar a minha mais profunda gratidão:

Esta monografia não teria sido possível sem o apoio científico e a orientação da Senhora Professora Doutora Maria Alexandra Brito. Muito obrigado por todos os ensinamentos, cuidado e atenta supervisão ao longo deste período.

À minha família: a eles devo tudo o que sou. Aos meus pais André e Elisabete pelo apoio, amor e confiança. À minha irmã Maria Laura e ao meu cunhado Fábio pelo carinho, ajuda e dedicação ao longo dos anos e principalmente por me terem presenteado com o melhor que uma tia poderia desejar, Ana Laura e João Pedro. À minha tia Kika por ser um exemplo de força e perseverança. Obrigada por terem acreditado em mim.

À maravilhosa família pela qual tive a sorte de ser presenteada, Sr. Armando Rosa e D. Maria do Rosário. Obrigada por me tratarem como uma filha e sempre me apoiarem.

Aos meus amigos: Camila Leite, Agostinho Lemos, Ana Rita Neves, Joana Moreira. O meu sincero obrigado por toda a ajuda, pelas conversas e incansável apoio para que desse certo. A todos os meus colegas da FFUL, Dany Figueiredo, José Pio, Cláudia Afonso, Leida, Albertina, Kátia e Edirvânia que muito me ajudaram. Não podia também deixar de lembrar os meus amigos que mesmo estando no Brasil sempre me apoiaram e incentivaram neste percurso.

Por fim, ao Armando Rosa, que aguenta o meu stress mais do que ninguém, agradeço pelo carinho, cuidado, incentivo, por ouvir as minhas reclamações e por me aguentar todos os dias.Obrigada por acreditar sempre em mim e, principalmente, pelo amor, ajuda e dedicação.

"Quando penso que cheguei ao meu limite,

descubro que tenho forças para ir além"

Ayrton Senna

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ABBREVIATIONS

Alzheimer's disease	AD
Amyotrophic lateral sclerosis	ALS
Blood brain barrier	BBB
Central nervous system	CNS
Converting enzyme, cyclooxygenase-2	COX-2
Cerebrospinal fluid	CSF
Chemically modified tetracyclines	CMT
Doxycycline	DC
Food and Drug Administration	FDA
Inducible nitric oxide synthase	iNOS
Interleukin-1	IL-1
Major histocompatibility complex II	MHC II
Matrix metalloproteinases	MMP
Minocycline	MC
Mitogen-activated protein kinase	MAPK
Mixed lineage kinase domain-like protein	MLKL
Multiple sclerosis	MS
N-*methyl-D-aspartate*	NMDA
N,N-dimethylglycylamido	DMG
NOD-like receptor protein 3	NLRP3
Parkinson disease	PD
Poly [ADP-ribose] polymerase	PARP
Protein kinase C	PKC
Reactive nitrogen species	RNS
Reactive oxygen species	ROS
Receptor-interacting protein kinase	RIPK
Structure activity relationship	SAR
Subthalamic nucleus	STN
Tumor necrosis factor receptor	TNFR

1. INTRODUCTION

The natural or semisynthetic tetracyclines had been stigmatized by the frequency of resistant microorganisms. However, they have been reborn at the time of recovering sensibility and incorporating new and more active components, which is why this review is made on the most outstanding aspects in this matter. The most important aspects will be described, from their discovery to their structural characteristics, antimicrobial activity, drug interactions and their use beyond antibiotic therapy, emphasizing the neurological area.

1.1. The discovery of tetracyclines

Tetracyclines were discovered as natural products from actinomycetes soil bacteria from *Streptomyces aureofaciens*, a group of fungi also called ultra-molds, and these products were first reported in the scientific literature in 1948 by Dr. Benjamin Duggar worker at Lederle Laboratories[1,2]. He discovered the first compound belonging to the tetracycline's family, the chlortetracycline or the Aureomycin. Consequently, tetracyclines were noted for their broad spectrum antibacterial activity and in December 1st of 1948, Aureomycin was approved by the Food and Drug Administration (FDA) for clinical use and commercialized with clinical success beginning in the late 1940s to the early 1950s, where the scientific methods of microbiology and organic chemistry were being developed[2,3].

After this discovery, Pfizer Laboratories also joined the studies and Alexander Finlay had gathered thousands of soil samples from around the world, and isolated the soil bacterium *Streptomyces rimosus*[2]. Their organism produced a compound with similarity in color to Aureomycin, but it was slightly more water soluble and had better bioactivity, giving it a medical and competitive edge over Aureomycin in the treatment of infectious diseases, the compound was named oxytetracycline or Terramycin[4,5]. That was also clinically approved by FDA in 1950 and was competing with Aureomycin due to the better bioactivity in the treatment of infectious diseases[3]. However, at that time the chemical structure of both compounds was still unknown[5].

It was in 1952 that collaboration between scientists at Pfizer Laboratories and the chemist Robert Woodward from Harvard University, gave rise to Pfizer-

Woodward team. They postulated that both compounds possess a naphthacene core with four fused rings (named DCBA)with similar functional groups[3,5]. These natural tetracyclines, obtained from strains of *Streptomyces*, belonged to the first-generation tetracyclines[3].

A series of new compounds, obtained from the previous ones, present greater liposolubility and half-life, as well as better intestinal absorption. These compounds were classified as second-generation tetracyclines and included the doxycycline (DC) and minocycline (MC).[4]

The discovery of DCBA naphthacene core was followed by the emergence of second-generation semisynthetic analogs and third-generation compounds, representing the evolution of the tetracycline scaffold concerning derivatives with increased potency and efficacy against tetracycline-resistant bacteria and improved pharmacokinetic and chemical properties. In 1954, the third compound was approved by FDA for clinical use – tetracycline – as the first novel tetracycline by modification of a natural product. Further molecular modifications in the tetracycline nucleus were applied to fulfil the generation of more potent and active tetracycline antibiotics. Working independently Pfizer Laboratory discovered in the following order the methacycline, precursor of DC an analog with remarkable activity, stability, and pharmacological efficacy, that was approved for human use as antibiotic by the FDA in 1967[2], whereas Lederle Laboratory discovered demeclocycline, the sancycline and finally the MC.[2,4,6]

The compound named MC was approved to use in 1971 after a long pathway of modifications of the analogs. MC, the most powerful tetracycline of that period, became one of the most widely used compound of tetracycline's family, and was the last tetracycline to be introduced into the market in the 20th century[6]. MC is a semisynthetic second-generation tetracycline that exerts anti-inflammatory effects that are completely separate from its antimicrobial action[7], observed firstly in 1983 by Lorne Golub and Nungaravam Ramamurthy. It was further described that the tetracyclines affect matrix metalloproteinases (MMPs) enzyme activity in cells, where they can act upon specific isozymes expressed over different inflammation-based disease states[2].

Finally, the most recent and powerful components of this family are glycylcyclines. These derivatives of MC, were classified as third-generation of tetracyclines[5,8].

1.2. Tetracyclines and their derivatives

The tetracyclines can be divided in three groups: natural products – first generation compounds (chlortetracycline, oxytetracycline, tetracycline, demeclocycline). Semisynthetics – second generation of tetracycline derivatives (metacycline, DC, MC) and chemically modified tetracyclines (CMTs) - third generation derivatives (limecycline, rolitetracycline, tigecycline, PTK 0796) derived from different species of *Streptomyces spp*[4,9] (**Table 1**).

Tetracycline semisynthetic second-generation compounds include DC and MC. They are currently used as antibiotics in humans[10] and exhibit higher antimicrobial activity than tetracycline against both gram-negative and gram-positive bacteria, even against some tetracycline-resistant strains. MC has circa twenty-fold higher affinity to ribosomes than tetracycline. However, MC has around five-fold lower affinity than the third-generation compound tigecycline. Besides MC affinity to ribosomes, the inhibition of *in vitro* translation by MC is more efficient than by tetracycline.

Table 1. Main componentes of the tetracycline group

Generation	Generic Name	Product Characteristics
First (1948 – 1963)	Chlortetracycline	Produced by two different species of Streptomyces discovered at the end of 1940's.
	Oxytetracycline	Obtained from Streptomyces in the 1950's.
	Tetracycline	Semisynthetic derivatives characterized by their water solubility.
Second (1965 – 1972)	Doxycycline	Semisynthetic derivatives of the first generation.
	Minocycline	
Third (Since 1993)	Glycylcycline (Tigecycline)	Semisynthetic derivatives of minocyclines.
	Aminomethylcyclines (Omadacycline – PTK 0796)	PTK 0796 is under clinical trials

Adapted from Vicente, D. & Perez-Trallero, E.[11]

2. CHEMISTRY OF TETRACYCLINES

2.1. Structure-activity relationship

Tetracyclines share the commonstructure core composed of four fused rings[3,4] (**Figure 1**) All form chelating complexes with different cations, such as calcium, magnesium or iron, which makes them insoluble in water, hindering their absorption.

Chemical modifications of first- and second-generation tetracycline derivatives produced both active and inactive compounds, leading to a general characterization of the structure activity relationship (SAR) for tetracycline activities[2].

The basic tetracycline structure consists of DCBA naphthacene core containing four fused aromatic rings. The tetracycline analogs differ only on the fifth, sixth, seventh or ninth position of the basic structure[4,6] . The DCBA structure has upper and lower peripheral zones with various chemical functional groups and substituents[12]. Chemical modification of C10, 11 and 12 abolishes bioactivity[2,4]. C4 with the natural isomer 4*S* is crucial for optimal antibacterial activity and epimerization to its 4*R* isomer decreases activity in gram-negative bacteria[2], as in CMTs tetracyclines, but CMTs still have the ability to bind other non-microbial targets as MMPs, giving them capacity to be used in the treatment of other diseases[13]. If lower peripheral region is modified, both antibiotic and non-antibiotic properties are reduced[12,13], due to the lack of site for metal ion chelation[13]. However, if upper peripheral region is modified, particularly in C7 to C9 positions, biologic activity of the compound may increase[7,12], which was the basis for the obtained higher efficacy with the semisynthetic compounds MC and DC[14].

The compounds tetracycline and MC have a similar chemical structure and it seems that they bind analogously to ribosomes. The difference that confers more efficiency to MC resides in the C7-dimethylamido group on ring D. Moreover, second-generation tetracyclines are more lipophilic and can be more easily absorbed[4]. MC has a longer half-life than tetracycline and better tissue penetration and has almost 100% bioavailability[7,14].

The CMTs, including the glycylcyclines possess a *N,N*-dimethylglycylamido (DMG) moiety on the C9 position of ring D. The 9-DMG derivatives presented a higher inhibitory activity in a large spectrum of gram-negative and gram-positive bacteria in comparison with first- and second-generation tetracycline derivatives.

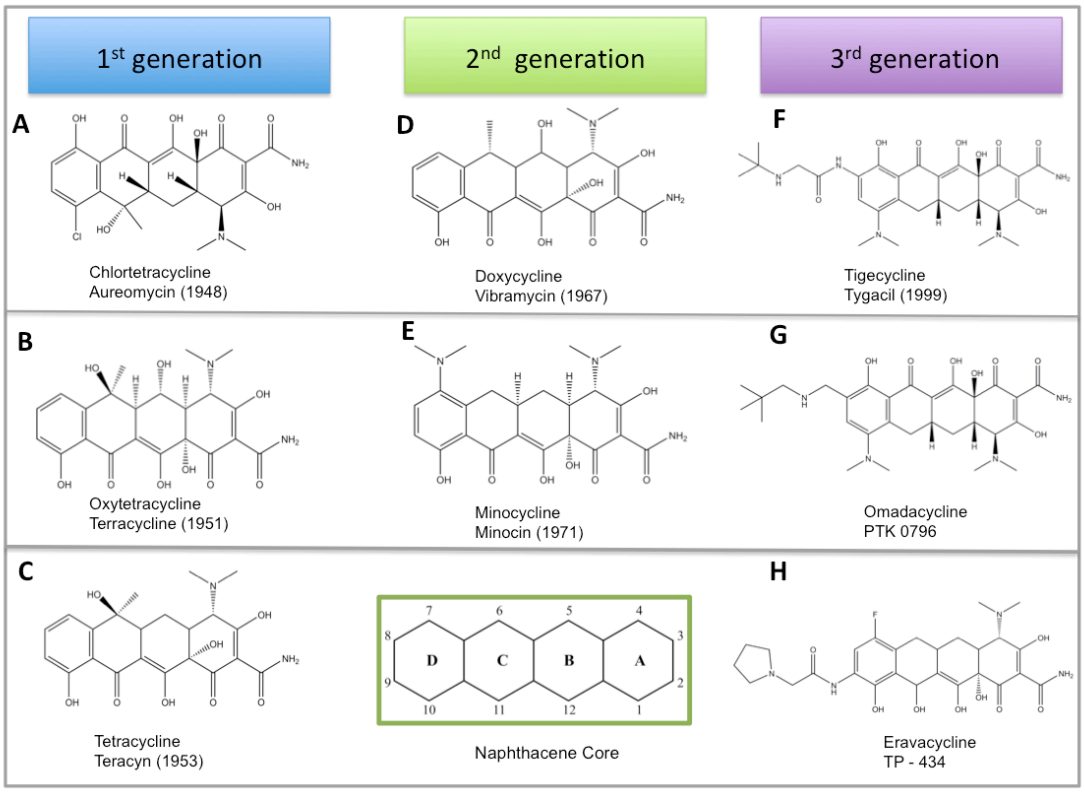

Figure 1 – Chemical structures the tetracyclines.

Chemical structures of the first-generation tetracyclines **(A–C)**: **(A)** chlortetracycline (Aureomycin®); **(B)** oxytetracycline (Terracycline®); and **(C)** tetracycline (Teracyn®). Second generation tetracyclines **(D–E)**: **(D)** doxycycline (vibramycin) and **(E)** minocycline (Minocin®). Third generation tetracyclines **(F–H)**: **(F)** glycylcycline tigecycline (tygacil); **(G)** aminomethylcycline omadacycline (PTK 0796); and **(H)** fluorocycline eravacycline (TP-434). The numbers in parentheses indicate the year of antibiotic discover/report. The inset of DCBA naphthacene core provides the carbon atom assignments for rings **A–D**. Adapted from Nguyen, F. *et al.*[4]

3. ACTION OF TETRACYCLINES AS ANTIBIOTICS

The antibiotic effect of tetracyclines has been widely studied and demonstrated over the years. Their great therapeutic application in both animals and humans is justified by their favourable antimicrobial properties and by the scarce presence of serious adverse effects[15].

The mechanism of action of these compounds consist in the inhibition of the synthesis of bacterial proteins by binding to the ribosomal subunit 30S of the bacteria. They are basically bacteriostatic agents, with activity against a large variety of microorganisms, by what became habitual antibiotics in humans, animals, and also used in some areas of agriculture[9].

For the tetracyclines to exert their action at the level of ribosome of gram-negative bacteria, it is required that they penetrate the cell of the microorganism by mechanisms of passive diffusion through the hydrophilic channels (porins) and by active energy-dependent transport processes[4,16].

During passive diffusion, a molecule simply dissolves in the phospholipid bilayer, diffuses across it, and then dissolves in the aqueous solution at the other side of the membrane. Once inside the cell, the tetracyclines bind reversibly to the receptors in the 30S subunit of the bacterial ribosome and in this way the binding of the aminoacyl-tRNA in the mRNA-ribosome complex is blocked[4,16].

Tetracycline penetration mechanisms are less specific for gram-positive bacteria. The selectivity of tetracyclines to inhibit protein synthesis in the bacteria lies in the fact that mammalian cells lack the active transport system and, in addition, the characteristics of the bacterial ribosome are different from those of mammalian ribosome[17].

Tetracyclines present an alternative mechanism of action: they can act as bactericidal agents, affecting cellular growth by membrane-mediated mechanisms: they may alter the cytoplasmic membrane of bacteria and release the intracellular contents from the cell[2].

Not all tetracyclines act similarly against bacterial ribosomes, where MC proved to be the most potent inhibitor of protein synthesis and tetracycline the least one[2].

When the different types of resistances were emerging and spreading between numerous bacterial species (efflux, enzymatic inactivation, ribosomal protection), the indications of the tetracyclines were being reduced to some empirical indications and to infections documented by antibiogram[18,19].

4. TETRACYCLINES BEYOND THE ANTIBIOTIC

The applications of these compounds outside the scope of the microbiology have been focused in the fact that tetracyclines exhibit anti-inflammatory and anti-apoptotic effects and affect multiple processes such as angiogenesis, proteolysis and bone metabolism[7,15] (**Figure 2**).

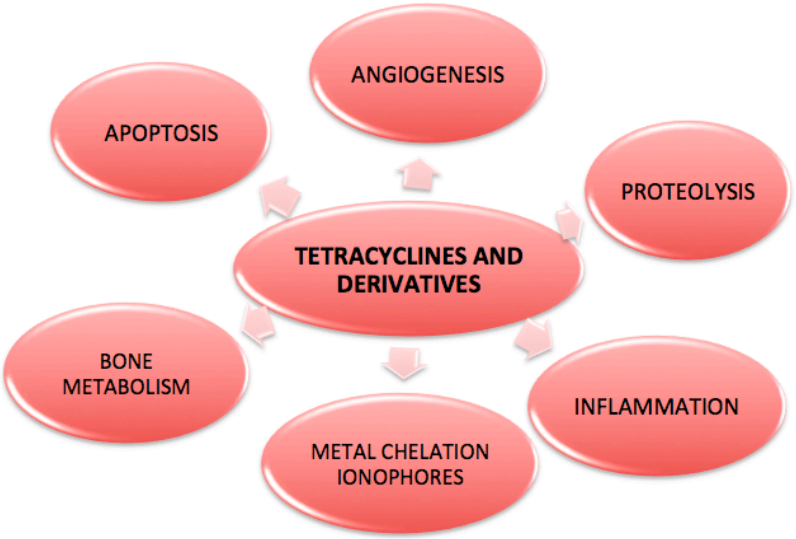

Figure 2 – Non-antimicrobial properties of tetracyclines and their derivatives.

Tetracyclines also present other properties that make them useful in various fields of the biomedical area[15]. Anti-inflammatory and immunomodulatory properties have been evidenced, and they include various mechanisms among the peripheral organs and the cental nervous system (CNS)[12,20]. These properties give tetracyclines different abilities: inhibition of inflammation; inhibition of proteolysis (inhibition of MMPs, involved in the rheumatoid arthritis, remodeling of the connective tissue and so in the tumor invasion and metastasis, among others); influence on bone metabolism; inhibition of angiogenesis that occur in many diseases and where MMPs are involved; anti-apoptotic properties by inhibiting caspase-1, a crucial protease in the regulation of mammalian apoptotic cell death, resulting in reduced tissue injury and neurologic deficits; and acting as metal chelation ionophores, organic compounds that form lipid-soluble complexes with metal cations, generally Ca^{2+} and Mg^{2+}, and transportable across hydrophobic barriers, affecting pathways as cell division and metabolic reactions[7,12,13].

Numerous studies are being carried out to find new practical applications of tetracyclines. Nowadays, the only noninfectious indication is to limit the loss of periodontal tissue in chronic periodontal diseases by DC[21].

One of the most studied actions of tetracyclines is their ability to inhibit MMPs. These are a group of proteolytic enzymes involved in a wide variety of pathological processes. Their presence in tumors and metastases as well as in inflammatory

processes, make this enzyme group the object of research mainly in the area of oncology for the development of new treatments[22]. MMPs are a family of zinc- and calcium-dependent endopeptidases responsible for remodeling and degradation of the extracellular matrix. This proteolytic group has many structural and functional characteristics in common but may differ both in the cellular origin and in the specificity by the substrate. As an example, the MMPs synthesized in connective tissue cells are involved in physiological processes of healing and bone resorption[23].

5. EFFECTS OF TETRACYCLINES IN THE CNS

Tetracyclines prevent cell death by at least two mechanisms: attenuation of innate and adaptive immunity and blocking of apoptotic cascades[10,13]. These mechanisms lead to an anti-inflammatory effect and cell protection, in general[14] (**Figure 3**).

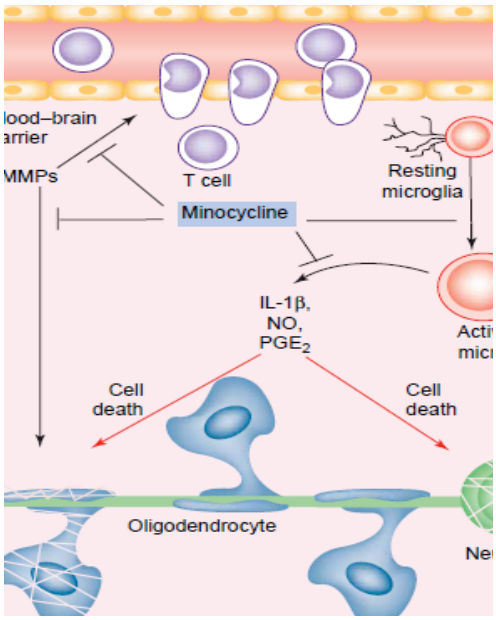

Figure 3 - Mechanisms of neuroprotection by tetracyclines. Tetracyclines attenuate both innate and adaptive immune responses. Thus, tetracyclines reduce microglial activation and thereby reduce transcription of the downstream pro-inflammatory mediators caspase-1, inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 and the subsequent release of interleukin 1β (IL)-1β, nitric oxide (NO) and prostaglandin E_2 (PGE_2), which are associated with cell death. Tetracyclines also inhibit the expression and activity of MMPs, which regulate BBB permeability and, consequently, peripheral cell infiltration and ensuing demyelination. Activated MMPs can also directly contribute to myelin degradation by myelin basic protein (MBP) digestion. Reproduced from Domercq, M. & Matute, C.[10]

CNS is a system that is immune-mediated by microglia. The activation of microglia leads to the release of pro-inflammatory mediators and other injury response factors that can influence neuronal and oligodendroglial viability.

Besides, MMPs can alter the blood-brain barrier (BBB) permeability which enables the T-cell migration into the CNS and subsequent myelin degradation, due to the effect of MC[10]. In the CNS, several studies have shown that tetracyclines are associated with the inhibition of pro-inflammatory levels of cytokine, which are interconnected with the referred MMPs, and reactive oxygen species (ROS), to block

neuronal and oligodendroglial cell death[10].

Apoptosis is characterized by having an intracellular proteolytic cascade mediated by two types of caspases: initiator caspases and executioner caspases. Apoptosis is a mark in neurodegenerative diseases, especially when involving mitochondria[24]. Tumor necrosis factor-α (TNF-α), ROS and reactive nitrogen species (RNS) are known to have an important role in disorganization of apoptotic pathways and in increasing caspase activity, being this way strongly related with the pathogenesis and pathophysiology of neurodegenerative disorders[25]. MC can act in various sites of apoptosis cascade to suppress apoptotic signals in cells, as illustrated in **Figure 4**[13].

MC has emerged as the most effective tetracycline derivative at providing neuroprotection, confirmed in experimental models of ischemia, traumatic brain injury, neuropathic pain and neurodegenerative conditions[7,14]. This compound prevents microglial activation and proliferation[7,25,26] (this process has deleterious effects on neurogenesis and neuronal survival)[7] without affecting astrogliosis, inhibits apoptosis and inflammation as mentioned earlier and inhibits oxidative stress[10,20]. It seems that the anti-inflammatory property of MC, especially its ability to block the activation of microglia is one of the mechanisms responsible for its cytoprotective properties of SNC[27,28].

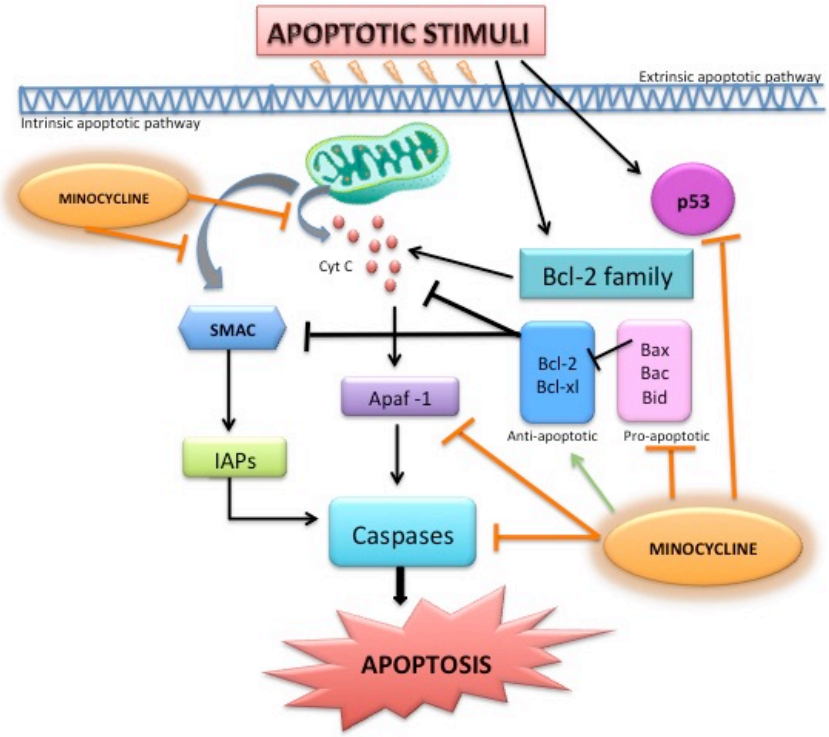

Figure 4 – Possible sites of action of minocycline on suppressing apoptotic signals in cells.

Minocycline interferes with different molecular elements in the programmed cell death pathway. Thus, minocycline inhibits the release of the pro-apoptotic factors: apoptosis-inducing factor, SMAC and cytochrome *c* from mitochondria by controlling mitochondrial permeability. Minocycline upregulates Bcl-2, which antagonizes the death-promoting factors Bax, Bak and Bid (inducers of cytochrome *c* release). Furthermore, minocycline also inhibits the activation of caspase-1, preventing cleavage of Bid to tBid. Abbreviations: APAF1, apoptosis protease-activating factor 1; IAP, inhibitor of apoptosis.

5.1. Blood-brain barrier

The internal environment of the CNS is isolated from blood circulation through the BBB. The BBB can be defined as a functional property of blood vessels of the CNS whereby the free exchange of solutes between plasma and nervous tissue is limited. So, except water, soluble and small gases liposoluble molecules (400-600 Da), the rest of organic molecules cannot freely cross the endothelium, but through specific transport systems[29].

Endothelial cells of the brain play a role fundamental in BBB, being able to highlight several characteristics that differentiate this capillary structure from the one existing in the rest of the vascular tree[29,30].

Specialized endothelial cells outline the wall of the brain capillaries and create a block constituted by adherence and tight junctions, which limit the flow of water, ions and large molecules into the brain. Pericytes envelop the brain endothelial cells in a non-symmetric pattern, contributing to the BBB properties and functions as a barrier. These pericytes and the endothelial cells are supported by astrocytes. All of the cells interact with each other and constitute the neurovascular unit[31].

As referred previously, although other tetracyclines can diffuse across the BBB and into the CNS in small amounts, MC is a small (495 kDa), highly lipophilic molecule capable of crossing the BBB and of being accumulated in the human CNS and in the cerebrospinal fluid (CSF), exhibiting a better efficiency than the others, for example in the treatment of many CNS diseases[4,7,14,24,32]. MC has demonstrated to regulate the BBB disruption by inhibition of MMP-9 and hypoxia-inducible factor-1α (HIF-1α)[33,34]. Moreover, a study with rodents showed that MC conferred protection against apoptosis during inflammation associated with brain edema and BBB dysfunction of the early brain injury[26].

6. TETRACYCLINES AND NEURODEGENERATIVE DISEASES

Recently, MC has been found to present properties potentially useful for the treatment of autoimmune disorders[7,12]. Of particular interest is also the potential application of MC in neurologic conditions such as stroke[12], Parkinson's disease (PD)[13], Huntington's disease[13], amyotrophic lateral sclerosis[32], ischemia, multiple sclerosis, spinal cord injury and Alzheimer's disease (AD)[7,24,25].

MC can exert its neuroprotection function due to its various mechanisms and properties as a impairs immune cell function. The main essential properties of this compound are summarized in **Table 2**.

Table 2: Minocycline effects on immune cells and its proposed mechanisms of action.

Cell Type	Minocycline effect	Mechanism of action
Microglia	Inhibition of proliferation	
	Inhibition of activation	**NF-κB pathway:** -Prevention of IκBα degradation ; -Inhibition of NfKB–DNA binding; **MAPKs Pathway:** -Inhibition p38 activation; -Inhibition of ERK1/2 and JNK1/2 activation
	Reduced production of proinflammatory mediators	
	Reduced antigen presentation capacity (MHC II expression)	Inhibition of PKCα/βII activation
T cells	Inhibition of proliferation	
	Inhibition of activation: -Reduced increase in cell size -Reduced T cell turnover -Reduced cytokine production -Reduced surface markers expression	Suppression of NFAT activation Attenuation of intracellular calcium signaling
	Reduced interaction with microglia	Down-regulation of CD40L expression
Monocytes/Macrophages	Inhibition of proliferation	
	Partial inhibition of activation: -Increased cytokine (IL-1β, TNFα, IL-6) production -Reduced production of proinflammatory mediators (derived from iNOS, MMPs and COX-2 activities)	
	Impaired antigen presentation capacity: -Reduced MHCII expression -Reduced antigen processing	Reduced PKCα phosphorylation
Neutrophils	Reduced chemotaxis	Inhibition of ICE
	Reduced MPO release	
B cells	Suppression of IgE responses	Inhibition of p38 MAPK in T cells

Adapted from Garrido-Mesa, N., Zarzuelo, A. & Galvez, J.[14]

Modification of neuron physiology: MC was demonstrated to attenuate this phosphorylation by Aβ and consequently to reduce neuronal cell death, improve cognitive impairment and attenuate the deficits in learning and memory [14] (**Figure 5**).

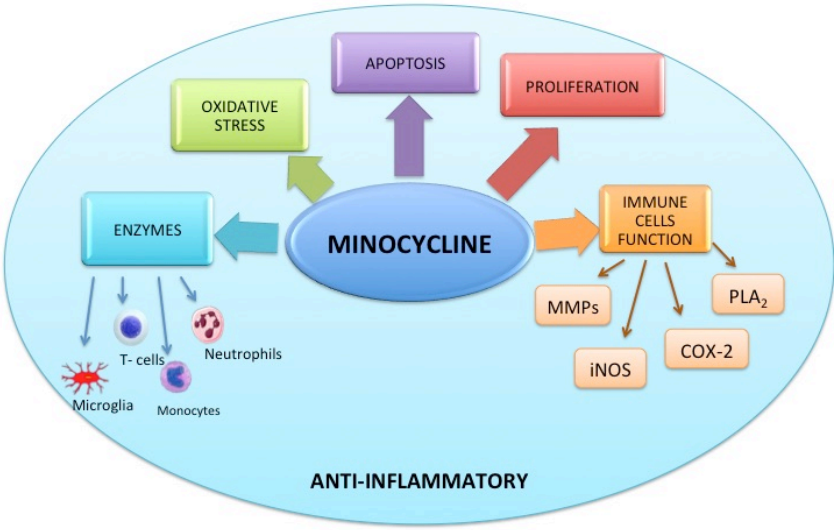

Figure 5 – Mechanisms involved in the anti-inflammatory activity of minocycline: inhibitory effects on enzyme activities, like iNOS, MMPs, cyclooxygenase (COX)-2 or PLA_2; inhibition of apoptosis, through the inhibition of caspase-1 and caspase-3 activation and the enhancement of Bcl-2-derived effects; antioxidant properties and inhibition of immune cell activation and proliferation.

6.1. Ischemia

Ischemia is a cerebrovascular disease characterized by the infiltration of inflammatory cells in the ischemic brain[7] leading to mortality and morbidity all over the world[35]. Ischemia causes immediate primary damage to CNS and a subsequent secondary degenerative response, developed days post-injury. The secondary response is characterized by inflammation and delayed neuronal death[36]. Reperfusion is a necessary process to restore the blood flow and to reduce neuronal damage but it can lead to injury. This injury followed by cerebral ischemia may have several consequences such as disturbance of neuronal metabolism, apoptosis, leukocyte adhesion and infiltration, and the breakdown of BBB. The increasing BBB permeability and subsequent disruption in reperfused patients have been showed to be correlated. One of the causes to this easy breakdown is the alteration of the expression levels of the tight junctions between the cerebral epithelial cells[35].

DC has been reported as safe and inexpensive neuroprotective agent that could reduce injury provoked by reperfusion in the CNS by blocking leukocyte adhesion. A study with DC treatment against a type of ischemia (middle cerebral artery occlusion) in rat models showed significantly reduced BBB leakage and cerebral infarct volume. This semisynthetic tetracycline derivative can easily cross the BBB, as previously referred. This compound up-regulated the expression of tight junction protein claudin-5, occludin and zonulaa occludens-1 (ZO-1), reduced the levels of MMP-2 and MMP-9 and down-regulated the expression of protein kinase C delta (PKCδ). This suggests that the reduced damage in BBB is correlated with the reduced levels of MMPs, PKCδ expression and up-regulation of tight junction proteins[35].

Similarly to DC, MC has been reported to reduce reperfusion injury by inhibiting MMPs and microglia activity after ischemia episodes[37]. MC has also been associated with neuroprotection with prevention of tissue damage spread and enhancement of functional recovery by reduction of expression of inflammatory mediators in the brain[24,36], such as the inhibition of the induction of caspase-1 with consequent iNOS[36]. MC showed prevention of microglial activation in a gerbil model of forebrain ischemia, with reduction of the infarct size and increasing of survival hippocampal neurons, even after ischemia already occurred. These effects included a reduction in the interleukin-1β (IL-1β) converting enzyme, cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) mRNA levels in the brain regions that were affected. However, these effects appear to depend on MMPs inhibition since MC had a protection role against cerebral ischemia in wild-type mice but not in MMP-9-deficient mice[7].

A study with adult spontaneously hypertensive rats that had transient middle cerebral artery occlusion during 90 minutes evaluated the influence of a single dose of 3 mg/kg of MC administrated intravenously versus a control group (treated with a vehicle). MC reduced BBB permeability, up-regulated tight junction proteins such as ZO-1, occludin and claudin-5, reduced the levels of TNF-α and IL-1β, and increased the levels of the anti-inflammatory factors: transforming growth factor-β (TGF-β) and IL-10. The early treatment with MC against reperfusion injury showed neurovascular remodeling during stroke recovery by the reduction of the brain tissue loss, the enhancement of tight junction proteins expression and the neuroprotective phenotype alternative activation of microglia/macrophages[37], which is similar to the results obtained for DC. An additional advantage demonstrated in this study was the fact of

only a single dose was administered, avoiding the possible toxicity due to the dose amount or to the various administrations.

In vivo studies with rats subjected to ischemia-reperfusion injury described the cardioprotective effects of MC with the reduction of infarct size, MMP-9 activity and oxidative stress. Another study also proposed poly(ADP-ribose) polymerase (PARP-1) inhibition as a possible mechanism for this protection given by MC[7].

Patients with stroke were evaluated and referred to MC treatment, after an experiment using mice with stroke where this compound have showed attenuation of infarct volume and neurological deficits due to the reduction of BBB disruption and hemorrhage. These patients were orally administrated with MC (200mg) for 5 days with a therapeutic window of 6-24h after stroke onset and the results showed a better outcome when compared with patients that were orally administrated with placebo[7].

MC has shown both anti-inflammatory, anti-apoptotic and neuroprotective effects against many models of cerebral ischemia, varying from the inhibition of MMPs to the good safety profile the compound presents and the high penetration in BBB[37,38].

6.2. Multiple sclerosis

Multiple sclerosis (MS) is a chronic autoimmune and neurodegenerative disease that affects the central nervous system, of idiopathic origin, that involves the loss of oligodendrocytes, myelin and axons, with an elevated expression of MMPs. These proteinases permeabilize BBB and allow the entry of T-cells into the brain with myelin degradation [32,36,39], as referred previously. With regard to injuries, these can occur in diverse locations of the CNS, causing different symptoms and cumulative deficits that give rise to a pattern of complex disability[40,41].

As occurs in other chronic pathologies of inflammatory origin, the clinical manifestations of this disease are variable. These can range from symptoms of benign disease to progression to more aggressive manifestations, which can cause the patient's disability and even death. Due to the fluctuating and irregular clinical course of this entity, the patient is led irreparably to the physical and cognitive deterioration of the sufferer [40,41].

The safety demonstrated over the years in clinical practice benefits the

potential of MC for its development as a cytoprotective drug in the treatment of different neurodegenerative disorders in humans, such as multiple sclerosis[40]. In a clinical trial involving patients with relapsing-remitting multiple sclerosis, MC therapy reduced the average number of lesions detected with the use of gadolinium enhancement ("lesions that improve") in MRI by 84% during a trial period (P = 0.03)[42].

In another trial, it was shown that the total number of lesions that improved was 63% lower with the combined use of glatiramer acetate plus MC than with glatiramer acetate plus placebo, although the difference was not significant[43]. In a pilot study in which MC (100 mg twice daily) was administered to 10 patients with relapsing multiple sclerosis. The results show that not only improved clinical manifestations and magnetic resonance studies, but also several serum markers of the immune system[42]. These trials demonstrate that the risk of conversion from a clinically isolated syndrome to MS is significantly lower in patients receiving MC[42,44].

MC has a neuroprotective role in MS due to the inhibition of MMP-2 and -9 that reduces apoptosis and leukocyte infiltration into the brain[24,32,36]. The compound delays episode onset, preserves motor function, reduces the risk of relapse and reduces decline in brain lesion volume[24,36]. This brain size reduction is associated with reduced macrophage migration from the periphery to the CNS, which results in less infiltration of leukocytes in the parenchyma of the spinal cord and decreased microglia major histocompatibility complex II (MHC II) expression and proliferation[7]. Moreover, there is evidence *in vivo* that MC can mitigate pyroptosis by direct inhibition of PARP-1 activity, stabilizes endogenous nuclear factor (erythroid-derived 2) like 2 (Nrf2) by reducing its levels, important in the inhibition of ferroptosis development, and inhibits the development of lipid peroxidation[25]. In MS disease, MC in combination with other drugs appears to be more effective than using drugs alone. This combination decreases the disease severity, attenuates inflammation, demyelination and axonal loss [7].

The success of MC led this compound to be tested in humans and the results corroborated its beneficial effects and found it to be safe and well-tolerated. MC showed significant reduction of relapse rates, active lesions and local brain atrophy. Patients also responded with immune changes that may be desirable to control this disease [7].

Due to the low cost and safety profile of MC, the results of these clinical trials are intriguing, despite their limitations; they constitute a convincing tool for future

studies of the effect of this drug in patients with multiple sclerosis. Still the use of MC in this pathology cannot be widely recommended until its benefit is demonstrated in long-term clinical trials and with a greater number of clinical evidences[45].

6.3. Spinal cord injury

This neurodegenerative injury acts similarly to ischemia in the CNS[36]. Microglia activation is believed to trigger nociceptive hypersensitivity in this disease and this way MC can have a crucial role for treating this condition[7]. The initial trauma in patients with traumatic spinal cord injuries triggers rapid hemorrhage and consequent cell death, offering few opportunities for therapeutic intervention. Subsequently, cascades of secondary lesions occur, causing a generalized and persistent inflammation and a progressive loss of tissue. At this stage, the injuries can be even greater than the initial trauma. Currently, therapies that act by inhibiting the progression of the secondary lesion have been found, therefore they appear as a promising and clinically viable approach for the reduction of tissue damage and functional deficits in the patient suffering from a traumatic spinal cord injury[46].

The post-traumatic reaction that leads to the secondary injury is characterized by the inflammation mediated by cytokines, proteases, ROS, including inflammation and edema, hemorrhage, ischemia, exitotoxicity of glutamate, cellular damage of free radicals such as ROS, RNS and the influx of calcium. However, most of the currently available therapies only cover one or a few of these mechanisms of secondary injury, and in most clinical trials have been unsuccessful[43,46]. These mediators can contribute to the activation of executioner caspases and consequently apoptosis[39].

It has been reported that MC acts as an inhibitor of the expression and activity of several mediators of tissue injury, which significantly attenuates the activation of microglia, inhibits caspase-1 and caspase-3, which are involved in the generation of IL-1 and apoptosis, respectively[47]. It is believed that its anti-inflammatory and neuroprotective activities in this type of lesions are partially achieved through conserved mechanisms such as the modulation of mitogen-activated p38 protein kinase (MAPK) and the signaling pathways of phosphoinositide 3-kinase (PI3K / Akt) and inhibition of MMPs. In these cases, MC also directly inhibits phospholipase A2 (sPLA2), which participates in the conversion of amino acids into

leukotrienes and prostaglandins, the latter being potent mediators of inflammation and secondary injury after a traumatic spinal cord injury[43,47].

Studies demonstrated that treatment of mice with MC showed superior behavioural recovery than that treated with methylprednisolone (the approved treatment for acute spinal cord injury in humans). Moreover, MC treatment in rats inhibited the release of cytochrome *c* from the mitochondria, increasing significantly long-term hind-limb locomotion[7,32]. The neuropathic pain behaviour attenuation was also correlated with reductions in microglia and astrocyte activation in a rat model of thoracic spinal cord injury[7].

Because MC can address several secondary injury mechanisms, it is a very promising drug to be used as an effective therapy in patients with traumatic spinal cord injuries. However, due to the small amount of clinical trials available, it is necessary to carry out additional investigations to determine the optimal dose, the route of administration and the duration of treatment in order to obtain the greatest benefit for the patient[43,46,47].

6.4. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a disease of the CNS, characterized by a progressive degeneration of motor neurons in the cerebral cortex (upper motor neurons), brainstem and spinal cord (lower motor neurons)[32,39]. The consequence is a muscular weakness that progresses to paralysis, extending from one body region to another. It threatens motor autonomy, oral communication, swallowing and breathing, although the senses, the intellect and the muscles of the eyes remain intact. The patient needs more and more help to carry out the activities of daily life, becoming more dependent[48].

The needs of patients multiply with the evolution of the disease, so it is essential to coordinate all actions to work in the same line, avoiding contradictions and reinforcing good practices. Early attention to these patients contributes to their better education and that of their family members in order to prevent situations of deterioration and to face the process of dependence[48].

Similarly to multiple sclerosis, studies reported increasing expression and activity of NOD-like receptor protein 3 (NLRP3), IL-1β, IL-18, caspase-1 and

caspase-3 in the tissues of patients suffering from this disease[7,25]. This disease has been associated to up-regulated expression of iNOS and p38 MAPK[7].

Oxidative stress originates when inside the cell an imbalance occurs between the pro-oxidants and antioxidants in which the former prevail. The ROS including the superoxide anion, the peroxynitrite or the hydroxyl radicals, are produced in the chain of mitochondrial electron transfer or by a variety of enzymes such as monooxygenases and oxidized as xanthine-oxidase, NADPH oxidase and cytochrome P450 enzymes. There is a considerable number of studies demonstrating that the oxidative stress induced by an increase in the formation of ROS is one of the main causes of tissue damage produced both in ischemic processes and in various neurodegenerative diseases such as ALS[43].

Many of the protective effects of MC in this disease could therefore be explained by its antioxidant capacity through an inhibition of the production of ROS by four different mechanisms of action:

1) For its ability to trap free radicals comparable to the antioxidant α-tocopherol.

2) By a direct action on the enzymatic complexes that lead to the generation of ROS.

3) Through a direct effect on the expression of genes involved in the production of ROS (eg COX-2 and iNOS).

4) By blockade in the activation of microglia, since this, once activated, constitutes one of the main sources of production of ROS.

MC, however, does not modify the expression levels of two of the main antioxidant systems used by cells to block the overproduction of ROS such as glutathione and NADPH, which are reduced in different neurodegenerative disorders[42].

In the first phase II studies in which minocycline was used in patients with ALS, two different doses of MC were used, 200 mg/day for six months in 19 patients and 400 mg/day for eight months in 23 patients. Patients at the lowest dose, no significant adverse reactions were observed, whereas at 400mg/day, gastrointestinal complications, plasma urea nitrogen and transaminase levels increased significantly[48].

Studies have been conducted in mice with ALS, where it has been shown that MC delays the onset of the disease and extends survival in these mice, inhibiting the

release of cytochrome *c* mediated by mitochondrial permeability, demonstrating this fact in vivo at the cellular level and in isolated mitochondria. It is believed that due to the safety profile of this drug and its ability to penetrate the BBB, MC can be considered a novel therapy for the treatment of ALS[43].

Another study compared the safety of the combination of riluzole (the only drug used in ALS, with rather low benefits) and MC (100 mg/day) for six months in 10 patients. The combination was safe, but no significant differences were found in the ALS-Functional Rating Scale between the two groups. In any case, the initial studies have given way to phase III clinical studies with disappointing results, since not only did MC prove to be unfavorable, but it even negatively influenced the course of the disease[49].

6.5. Huntington's disease

This disease is an autosomal dominant inherited neurodegenerative disorder of the central nervous system that predominantly affects the basal ganglia with progressive generation of GABAergic medium-sized spiny neurons in the caudate nucleus and putamen[39]. A feature of this disease is the presence of cellular aggregates/inclusions but their origin regarding time and causes remain unclear[14].

This pathology is characterized by the association of motor disorders (choreic syndrome, dystonia, postural disorders that can cause falls, dysarthria and swallowing disorders), cognitive, psychiatric and behavioral disorders (character changes, depressive syndrome, sometimes psychotic disorders)[50].

The pathophysiology of this disease occurs as a result of a functional inactivation or a lesion in the subthalamic nucleus (STN), which leads to a decrease in the activity of the GPi/SNpr complex (internal pale balloon / nigra pars reticulata substance)[44]. The mechanisms involved in the neuronal death that have been suggested are the excitotoxicity, metabolic impairment and oxidative stress[39].

The use of tetracyclines in these patients has now been described. This is because a selective decrease in the number of cells of certain neuronal populations in neurodegenerative diseases has been observed. Such is the case of Huntington's disease, where neuronal losses in the striatum have been observed, and in a less selective way, the cells of the penumbra area are also affected[20]. Despite presenting

a quite different pathophysiology, the intracellular mechanisms responsible for neuronal death that surround at this selective decrease of neurons they appear to be the result of the activation of apoptotic processes.[10,20]

During the last years, the hypothesis that the MC could modulate some of the stages described in these processes has been formulated: activation, decision and execution. It is believed that the mutation of caspase sites prevents neurodegeneration and improves the disease phenotype, this has been demonstrated in mice of experimental models of EH. Studies indicate that MC inhibits caspase-dependent (such as Smac / Diablo and cytochrome C) and independent routes (such as the AIF apoptosis inducing factor) in R6 / 2 mice improving the disease phenotype and showing neuroprotective capacity.[51]

It has been suggested that MC decreases the proliferation and activation of rodent microglia cells, resulting in a significant blockage of the production of nitric oxide and prostaglandin E2, perhaps due to its inhibitory effect on the expression of iNOS and COX-2.[52]

It is believed that treatment with MC significantly reduces the activation of reactive microglia induced by lipopolysaccharide and limits the increase in mRNA of IL-1β and TNF-α.[53] In an immune inflammatory encephalitis model, MC reduced the release of TNF from activated oligodendrocytes and potentiated the release of interleukin-10, an anti-inflammatory cytokine. In J20 APP-tg mice, an animal model of EA, MC reduced the expression of CD11b and CD45. This decrease resulted in a lower microglial production of inflammatory autacoids (IL-1, IL-6 and TNF) and neuroprotective substances (neuronal growth factor, NGF).[54]

6.6. Parkinson's disease

PD is characterized by the loss of dopamine neurons in the substantia nigra pars compacta and consequent microglial activation and by progressive motor decline with subsequent cognitive and other deficits, which can lead to the impairment of activities associated with daily living[55].

Parkinson's patients do have plasma concentrations of inflammatory cytokines increased[55]. Despite the exact mechanisms that lead to this disease are not well understood, it is known that disruption of cell-autonomous processes

involved in modulation of protein folding and aggregation, and non-cell-autonomous processes involving mitochondrial function, oxidative stress and inflammation may be implicated[56].

Although tumor necrosis factor receptor 1 (TNFR1) activity is increased and TNFR2 activity is decreased in this disease, it is confined to the substantia nigra in the abnormal brain of these patients[7,57]. TNFR1 activity can be predictive of the development of neurocognitive disturbance in PD but there are no reported studies relating TNFR1 activity and the severity of motor symptoms. High levels of TNF-α is also present in the brain and CSF of these patients. In this disease, c-Jun N-terminal kinase (JNK) signaling is dysregulated, which will facilitate dopaminergic neuronal death and modulation of p53 upregulated modulator of apoptosis (PUMA) activity. Dopaminergic neuronal death in this pathogenesis can be due to apoptosis signaling kinase-1 (ASK-1) -JNK1-mediated or by lysosomal rupture. Besides, ferroptosis is an important mediator of cell death in this neurodegenerative disease[25]. Similarly to Huntington's disease, MC effects in PD are also associated with the reduction of iNOS and caspase-1 expression.[7]

Numerous investigations have demonstrated the beneficial effects of MC against different dopaminergic toxins; however, to date, only one phase II clinical study has been published in which it is concluded that MC is a good candidate for subsequent phase III trials. The objective of this study was to verify whether creatine and MC affected the course of early PD in relation to the predetermined threshold of uselessness for disease progression. The participants in this trial had an average of 5 years with the diagnosis; however they did not require medications to treat the symptoms. Patients received 10 g/day creatine, 200 mg/day MC or placebo depending on the group in which they were assigned. The tolerability of MC was 77% and 91% in the creatine group. The main adverse events presented included upper respiratory symptoms (26%), joint pain (19%) and nausea (17%), which is why it is necessary to consider MC for definitive phase III trials and thus determine whether or not it alters the progression of PD in the long term. However, it is also necessary to weigh additional factors such as the activity, tolerability, safety, cost and availability of these drugs before selecting them for phase III trials.[58]

For its part, the European Multiple System Atrophy-Study Group has completed a phase III study (ref.: NCT00146809), whose hypothesis was that treatment with 50 mg of MC twice a day could affect the progression of clinical symptoms and the diagnosis in patients with multisystem atrophy, however the

results of this study to date have not been published.[59]

6.7. Alzheimer's disease

AD is progressive neurodegenerative pathology characterized by the presence of cognitive impairment, deficits in learning and memory loss, two of the most important neuropathological features are lysosomal rupture with formation of amyloid plaques and the formation of neurofibrillary tangles, caused by dysfunction and accumulation of amyloid-β peptide and abnormally phosphorylation of *tau* protein [25,60].

In the pathophysiology of this disease, the structural and functional integrity of the cerebral vasculature is involved, a critical element in maintaining homeostasis and therefore neuronal viability. In this sense, it has been suggested that the loss of the properties of the vasculature could contribute to the pathophysiology of some neurodegenerative diseases such as AD in which a poor cerebral flow, vascular inflammation, or dysfunction of the BBB have been described[61].

There is no treatment for AD. Currently, the general objectives of the treatment are aimed at encouraging the patient to stimulate or maintain their mental abilities, autonomy, and self-identity, improve their cognitive performance, delay cognitive impairment, and prevent mood disorders and other complications. In short, improve their quality of life and, with that, that of their family[62,63]. The treatment of AD can be non-pharmacological and / or pharmacological.

Regarding pharmacological treatment, the medications available to date do not produce improvements in cognitive functions, and only slow the progression of the disease, therefore they do not have an impact on survival, although they do improve the quality of life of patients[63]. There are several approved drugs for the treatment of AD whose mechanism of action is based on the modulation of cholinergic or glutamatergic neurotransmission, having shown positive effects on cognitive function, although its impact on the global situation of patients is modest and has questioned its cost-effectiveness[63].

Currently, only two types of specific drugs are available for the symptomatic treatment of AD: acetylcholinesterase enzyme (AChEI) inhibitors and a non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist. These current treatments do not work in all patients equally and are effective only for a short period

of time. Therefore, there is a clear need to develop drugs that extend this period[63].

It is believed that MC exerts certain protective effects on the vasculature, apparently due to an inhibitory effect on the expression and activity of MMP. As mentioned above, MMPs are the enzymes responsible for the replacement of the extracellular matrix and the degradation of proteins that in pathological situations can alter the permeability of the BBB, enabling the migration of T lymphocytes to the CNS and subsequently the degradation of myelin[64].

A recent article indicates that the inhibition of caspases 3 and 7, important apoptosis-executing molecules, blocks the overactivation of microglia thus causing neurotoxicity, these results revitalize the interest in caspase inhibitors as potential therapeutic agents in CNS diseases that occur with neuroinflammation and glial overactivation[65,66].

The direct effects of the activation and inhibition of microglia on the clearance of peptide A by inactivating microglia with MC have also been studied, demonstrating the role that these cells play in the pathogenesis of the disease, which make it possible therapeutic targets[67].

Other studies indicate that treatment with MC is capable of reversing the aversive and spatial damage caused by the administration of Aβ1-42 oligomers in Balb/c mice, an animal model of Alzheimer's-type dementia[68]. It is believed that one of the mechanisms by which MC acts against the memory damage of these animals is through the reduction of the levels of inflammatory cytokines in the total cortex, the hippocampus and the serum[64,68].

The restoration of Neurotrophin levels in the total cortex and hippocampus is another mechanism that probably contributes to the improvement of the memory of these mice after receiving treatment with MC. Therefore, neuroinflammation is a mechanism consistent in the pathology of AD. These findings reinforce the anti-inflammatory effects of MC, as well as its effects on cognition and neurotrophins.[68]

It has also been found that MC is able to reduce the level of Aβ1-42, reducing microglial activation, and the content of TLR2, a receptor that appears to be activated by Aβ1-42, since there is an increase in this in the hippocampus of mice injected with these oligomers.[69] MC also reduces levels of MyD88 protein, TLR2 receptor adapter molecule, responsible for signal transduction from the receptor. This reduction in TLR2 activation seems to have been responsible for the cytokine reduction caused

by the treatment with MC.[68,69]

For its part, for the maturation of IL-1β, its release in the extracellular environment and the spread of inflammation, the activation of the NLRP3 inflamasome is required. An increase in the content of NLRP3 has been found in animals administered with βA and that MC is able to reverse this increase, leading to a reduction in IL-1β and other inflammatory cytokines (**Figure 6**).[68,69]

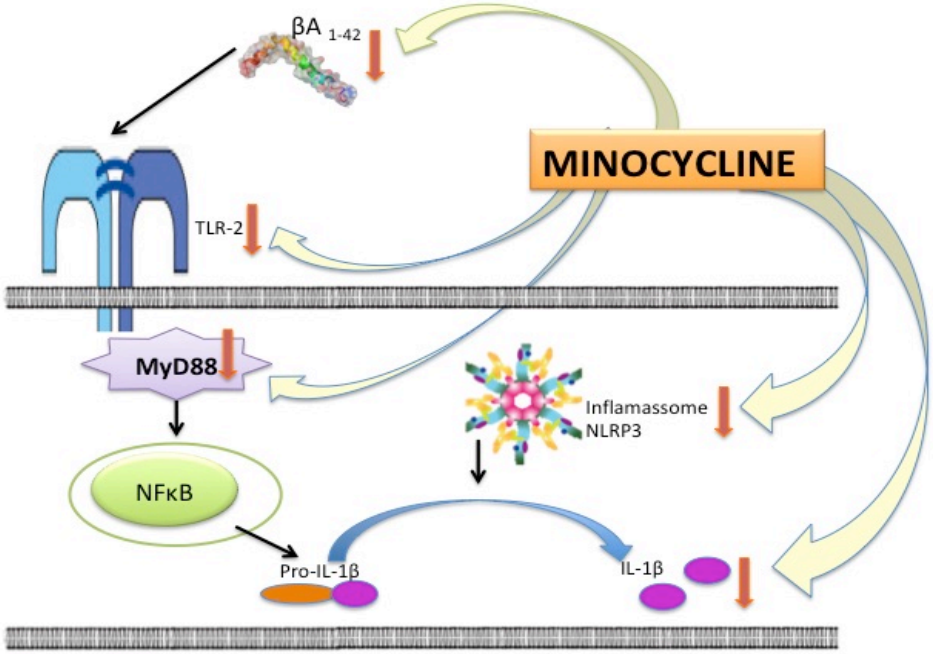

Figure 6: Mechanisms of action of minocycline through the release of IL-1β and other cytocytes and the restoration of neurotrophin levels. Minocycline reduced the content of βA, makes the TLR2 receptor of its molecule MyD88 and the NLRP3 protein constituent of the inflammasome, which is responsible for IL-1β cleavage and maturation. In addition, it reduced the levels of inflammatory cytokines (TNFα, IL-6, IL-4, IL-10) in hippocampi, total cortex and serum and BDNF and NGF in brain structures. Adapt from: Garcez, M. [68]

As can be seen, MC has proven to be a therapeutic alternative for the relevant anti-inflammatory action that occurs in AD, leaving high expectations in the future, however, it is necessary to carry out future clinical studies that confirm the benefits of MC for this disease. [64,68-70]

7. CONCLUSIONS

Tetracyclines are compounds used for antimicrobial and non-antimicrobial purposes. As an antibiotic, their mechanism of action is based on the linkage to the 30S ribosomal subunit in bacteria (both gram-negative and gram-positive) and the consequent blockage of protein synthesis, leading to cell death and inhibition of bacteria growth.

Although tetracyclines mechanism of action involved in the antibiotic properties is known, the exact mechanism of action that confers tetracyclines, and especially MC, the anti-inflammatory, immunomodulatory and neuroprotective properties in the CNS lacks more studies and deep knowledge. However, there are already guide lines about the mechanisms involved such as: i) inhibition of enzyme activities; ii) antioxidant properties; iii) inhibition of apoptosis pathway by inhibition of the activation of caspase-1 and -3; iv) enhancement of Bcl-2 derived-effects and consequent protection of cells against apoptosis; v) reduction of MAPK phosphorylation; and vi) inhibition of PARP-1[14].

Since MC has presented so many potential results against many models of neurodegenerative diseases, such as the anti-inflammatory and the anti-apoptotic effects, the fact of being known the side-effects profile, being considered a safe drug and inexpensive which allows the long-term use, and the high penetration in the BBB, makes this compound the ideal to use not only in stroke treatment but also in multiple sclerosis, spinal cord injury, amyotrophic lateral sclerosis, Huntington's disease, Parkinson's disease, Alzheimer's diseases. This compound can also be combined with other drugs to obtain a synergistic effect. Due to the vast mechanisms of action proposed for MC, this compound can also affect multi-targets.However, despite MC is considered the most effective tetracycline derivative regarding neurodegenerative diseases, there are still limitations to its potential: the dose administrated to avoid undesirable side-effects remains a crucial issue[36]. Besides, the mode of administration is also important to evaluate the effects, since MC has variable effects depending on this condition[7].

To conclude, of all the neurological diseases considered here, MC appears to have the most potential against multiple sclerosis. For this disease, both experimental studies with animals and phase I and II clinical trials in humans showed concordant results[7].

8. FUTURE PERSPECTIVES

The combination of MC with other drugs constitute a possibility to treat neurodegenerative disorders, for example the prion diseases, as proposed by the study with prion-infected hamsters[71]. Besides, DC has also been reported as a neuroprotective agent[35], and their combination to long-term uses could be a novel approach in oral administration.

Regarding MS treatment with MC, phase III clinical trials should be explored[7]. The similar is proposed to the spinal cord injury treatment, since MC treatment in humans is needed to evaluate and confirm the potential application demonstrated by animal experiments.

Additional studies should be performed in Huntington's and Parkinson's diseases to determine whether MC has a neuroprotective effect in those. Regarding Alzheimer's disease, more work should be done to determine MC usefulness in patients[7].

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