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The potential use of tetracyclines in neurodegenerative diseases and the role of nano-based drug delivery systems

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ABSTRACT

Neurodegenerative diseases are still a challenge for effective treatments. The high cost of approved drugs, severity of side effects, injection site pain, and restrictions on drug delivery to the Central Nervous System (CNS) can overshadow the management of these diseases. Due to the chronic and progressive evolution of neurodegenerative disorders and since there is still no cure for them, new therapeutic strategies such as the combination of several drugs or the use of existing drugs with new therapeutic applications are valuable strategies. Tetracyclines are traditionally classified as antibiotics. However, in this class of drugs, doxycycline and minocycline exhibit also anti-inflammatory effects by inhibiting microglia/macrophages. Hence, they have been studied as potential agents for the treatment of neurodegenerative diseases. The results of *in vitro* and *in vivo* studies confirm the effective role of these two drugs as anti-inflammatory agents in experimentally induced models of neurodegenerative diseases. In clinical studies, satisfactory results have been obtained in Multiple sclerosis (MS) but not yet in other disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), or Amyotrophic lateral sclerosis (ALS). In recent years, researchers have developed and evaluated nanoparticulate drug delivery systems to improve the clinical efficacy of these two tetracyclines for their potential application in neurodegenerative diseases. This study reviews the neuroprotective roles of minocycline and doxycycline in four of the main neurodegenerative disorders: AD, PD, ALS and MS. Moreover, the potential applications of nanoparticulate delivery systems developed for both tetracyclines are also reviewed.

1. Introduction

Neurodegenerative diseases are chronic and progressive disorders that lead to the destruction of neurons in the central nervous system (CNS) being the main cause of cognitive and motor disorders (Amor et al., 2014). Prominent examples of these diseases are Alzheimer's

Abbreviations: MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 6-OHDA, 6-hydroxydopamine; ALS–FTD, ALS with frontotemporal dementia; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; AFM, atomic force microscopy; APP, Aβ precursor protein; Aβ, beta–amyloid; BBB, blood brain barrier; BSCB, blood-spinal cord barrier; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; CMT-3, chemically modified tetracycline 3; cMHNP, chitosan-based MH-loaded NPs coated with tween 80®; CNTF, ciliary neurotropic factor; CIS, clinically isolated syndrome; DST, despair swim test; EDSS, disability status scale; DLS, dynamic laser light-scattering; EAE, experimentally-induced autoimmune, encephalomyelitis; FDA, food and drug administration; FTIR, fourier transform infrared; FTLD, frontotemporal lobar degeneration; GABA, gamma-aminobutyric acid; GDNF, glial cell line-derived neurotrophic factor; GSH, glutathione; hGDNF, human GDNF; nHA, hydroxyapatite nanoparticles; G6, hydroxyl-generation-6; D-Mino, hydroxyl-generation-6 poly amidoamine dendrimer–9-amino-minocycline conjugate; IFN-γ, interferon-γ; IL, interleukin; IBA-1, ionized calcium binding adaptor molecule 1; LPS, lipopolysaccharide; MMPs, matrix metalloproteinases; MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration; MHNP, minocycline-loaded nanoparticles; MAPK, mitogen-activated protein kinase; MS, multiple sclerosis; NPs, nanoparticles; NGF, nerve growth factor; NFT, neurofibrillary tangles; NTS, neurotensin; NO, nitric oxide; NF-Kb, nuclear factor Kappa B; NMR, nuclear magnetic resonance; NOESY, nuclear overhauser effect spectroscopy; DCNPopt, optimized doxycycline hydrochloride-loaded chitosan based and tween 80® coated nanoparticles; PD, Parkinson's disease; PLGA, poly D,L-lactic-co-glycolic acid; PAMAM, poly(amidoamine); PSM, polysialic-acid-based minocycline-loaded micelles; PLC, poly-ε-caprolactone; PPMS, primary progressive multiple sclerosis; Prp, prion protein; PRMS, progressive-relapsing multiple sclerosis; ROS, reactive oxygen species; RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; SCI, spinal cord injury; TST, tail suspension test; Tht, thioflavin T; TNF-A, tumour necrosis factor alpha; UBQLN2, ubiquilin 2; UPDRS, unified Parkinson's disease rating scale.

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disease (AD), Parkinson's disease (PD), Amyotrophic lateral sclerosis (ALS), and Multiple sclerosis (MS).

Neurodegenerative diseases are a serious threat to human health. These diseases are on the rise due to the increase in life expectancy (Heemels, 2016). Although the main causes and progression of each neurodegenerative disease are different, all of them share common features at the molecular level including neuroinflammation, accumulation of aggregated misfolded proteins, impairment of degradative processes such as autophagy and the ubiquitin proteasome system, oxidative stress, and impaired mitochondrial functions (Martinez-Vicente, 2017).

Great research efforts are being made to find new effective treatments for these pathologies. An interesting strategy is based on drug repurposing; that is the use of drugs that have already passed toxicity and clinical safety tests for new therapeutic applications (Bortolanza et al., 2018).

This is the case of tetracyclines, a family of antibiotics commonly used in clinical practice. Among them doxycycline and minocycline are being investigated in neurodegenerative diseases due to their safety profiles and ability to cross the blood brain barrier (BBB) (Airoldi et al., 2011). Tetracyclines have effects beyond their antibiotic properties which justify their potential effects on the CNS such as matrix metalloproteinases (MMPs) inhibition, reactive oxygen species (ROS) scavenging, antiapoptotic effects, anti-inflammatory effects, protein antiaggregation activities, and protection against mitochondrial dysfunction (Sapadin and Fleischmajer, 2006).

The two synthetic derivatives of tetracycline; minocycline and doxycycline have demonstrated excellent neuroprotective effects in experimental models without any significant signs of toxicity (Bortolanza et al., 2018). The neuroprotective effects of minocycline may be explained through multiple mechanisms such as inhibition of caspase-1, caspase-3, MMPs, p38 mitogen-activated protein kinase (MAPK), and inducible form of nitric oxide (NO) synthase (Garrido-Mesa et al., 2013). In addition, other studies have shown that minocycline can shift activated microglia/macrophages to an M2 phenotype, thereby reinforcing its neuroprotective potential (Ahmed et al., 2017).

Moreover, other characteristics of this class of drugs such as long elimination half-lives, mild side effects, and lipid solubility allowing adequate permeability across the BBB (Griffin et al., 2010), can be very valuable for their potential use in neurodegenerative diseases.

Although the properties of tetracyclines make them potential valuable candidates for their use in neurodegenerative diseases, the management of these diseases has always been limited due to failures in efficacy, long-term side effects and limitations in developing an ideal drug delivery system. Nanotechnology is an interesting approach when trying to minimize drug delivery problems and optimize therapeutic benefits of medications. Nanomaterials include several types of devices (nanoparticles, nanofibers, nanotubes, quantum dots, …) with broad applications in several biomedical fields the development of drug delivery systems (Masoudi Asil et al., 2020).

The purpose of this work is to review the studies performed on tetracyclines, especially minocycline and doxycycline in relation to their potential use in four of the most common neurodegenerative diseases: Alzheimer's disease, Parkinson's disease, Multiple sclerosis, and Amyotrophic lateral sclerosis. Moreover, the potential applications of nanoparticulate delivery systems developed for both tetracyclines are also reviewed.

2. Tetracyclines as anti-inflammatory agents in neurodegenerative diseases

Tetracycline derivatives due to their pleiotropic effects may be used as an alternative treatment in cases where inflammation plays a key role in disease progression (Socias et al., 2018). An outstanding and common effect among all tetracyclines is downregulating the expression of proinflammatory mediators, effect which is particularly marked for minocycline and doxycycline (Golub et al., 1998). Tetracyclines can also affect the innate and adaptive immune responses (Griffin et al., 2010), as experiments have shown that doxycycline and minocycline are effective in reducing inflammation of various origins (Bahrami et al., 2012).

Tetracyclines can affect inflammation and cell death in several ways thereby reducing inflammation and in turn cell death. In this regard, some of the potential beneficial effects of tetracyclines are reduction of microglial activation and proliferation (Yrjänheikki et al., 1998, 1999), inhibition of inducible NO synthase and interleukin IL-1 expressions (Tikka et al., 2001; Jackson-Lewis et al., 2002), and inhibition of p38 mitogen-activated protein kinase (MAPK) and NF-kB signalling pathways (Nikodemova et al., 2006; Santa-Cecília et al., 2016), processes which play a key role in regulating the expression of pro-inflammatory mediators. Moreover, recent evidence confirmed the coexistence of different mechanisms of action of tetracycline derivatives regarding their anti-inflammatory effects (Ferreira Junior et al., 2021). In this study, doxycycline and cemically modified tetracycline 3 (CMT-3) reduced oxidative stress and TNF-α release in activated microglia. They acted as anti-inflammatory agents by reducing microglial glucose accumulation thereby inhibiting NADPH synthesis. According to these findings the authors concluded that both compounds could be promising therapeutic agents in chronic CNS pathological conditions such as PD, where long-term neuroinflammation plays a key role.

In addition, by inhibiting the activity of the caspase-1 and -3 pathways tetracyclines can prevent cell death in the CNS (Bode et al., 2019). A study conducted by Bernardino et al. (2009) showed that doxycycline and minocycline were able to reduce in a dose-dependent manner the production of tumour necrosis factor alpha (TNF-α), IL-6, and IL-8 in both stimulated human monocytic cell line THP-1 and glial cells. In experimental models of traumatic brain injury, neuropathic pain, ischaemia, and different neurodegenerative disorders (ALS, Huntington's disease, PD, AD, MS, Spinal cord injury), minocycline has demonstrated to be the most effective tetracycline derivative regarding neuroprotective effects (Garrido-Mesa et al., 2013).

Doxycycline dosage can be adjusted depending on whether an antibiotic or an anti-inflammatory effect is sought. For instance, a dose of 200–400 mg/day is required for antibiotic effects although clinical trials have demonstrated that sub-antimicrobial doses ranging from 20 mg/day to 40 mg/day) can result in anti-inflammatory effects (Di Caprio et al., 2015).

3. Alzheimer's disease

Alzheimer's disease (AD) is the most prevalent of the neurodegenerative disorders with age being a major risk factor as incidence rates increase after the age of 60 (Herrup, 2010). It is the most common type of dementia with memory loss and cognitive impairment being the main accompanied complications of the disease (Ismail et al., 2011). The most significant pathophysiological features of AD are the extracellular accumulation of beta-amyloid peptide (Aβ1-42) and the formation of neurofibrillary tangles (NFT) (intracellular deposits of hyperphosphorylated tau protein) (Serý et al., 2013). Increased and impaired Aβ precursor protein (APP) function causes amyloid beta plaques to accumulate forming the so-called "senile plaques". These toxic and insoluble plaques lead to neuroinflammation by activating the innate immune system, a process which leads to synaptic disconnection (Buoso et al., 2010; Heneka et al., 2014).

Inflammation plays a key role in the pathogenesis of AD (Ferreira et al., 2014). Inflammatory markers including cytokines are augmented in the brain of AD patients, especially in the entorhinal cortex and hippocampus (Czirr and Wyss-Coray, 2012). Moreover, blood levels of several inflammatory mediators are also increased. For instance, increased levels of TNF-α, IL-6, and IL-1β have been described in AD patients (Swardfager et al., 2010). Some studies have related the cognitive decline occurring in AD with augmented cytokine levels (Harries et al., 2012). Increased cytokines are also associated with high

levels of phosphorylated tau protein and decreased levels of synaptophysin, which in turn lead to changes in the synaptic function of patients suffering from AD (Quintanilla et al., 2004). In pro-inflammatory conditions, glial cells in the brain, especially astrocytes and microglia, become active resulting in reactive astrocytes and activated microglia surrounding the amyloid plaques (Thameem Dheen et al., 2007). Considering the pathogenesis of AD and the fundamental role of inflammation in its development and progression, it can be hypothesized that tetracycline derivatives exerting anti-inflammatory actions could potentially have a beneficial effect on the disease.

Preclinical studies performed both *in vitro* and *in vivo* have shown that tetracyclines have anti-amyloidogenic effects on a variety of amyloidogenic proteins. To demonstrate the mechanism of action of tetracycline on Aβ1–40 and Aβ1–42 at both molecular and supramolecular levels, Airoldi et al. (2011) carried out a series of experiments using NMR and FTIR spectroscopy, dynamic laser light-scattering (DLS) and atomic force microscopy (AFM). Surprisingly, this study explained a new aspect of the mechanism of anti-amyloidogenic activity of tetracycline resulting from hydrophobic and charged multiparticle interactions. They showed that co-incubation of Aβ1–42 oligomers with tetracycline prevented the toxicity towards mouse neural crest-derived cell line in a dose-dependent manner. In nuclear overhauser effect spectroscopy (NOESY) the spectra obtained did not show intermolecular cross-peaks between tetracycline and Aβ. For this, the absence of specific binding sites suggested the presence of supramolecular interactions. Furthermore, DLS and AFM studies supported this hypothesis as co-dissolution of Aβ peptides and tetracycline triggered the immediate formation of new aggregates that improved the solubility of Aβ peptides, inhibiting the progression of the amyloid cascade. NMR data showed that tetracycline competed with thioflavin T (ThT) for binding to Aβ peptides.

In a study carried out by Cai et al. (2013), the neuroprotective effects of minocycline were evaluated in diabetic rats. This metabolic disorder may play a role in the pathogenesis of AD by increasing the expression of both Aβ and tau proteins. In the study, the effect of minocycline on Aβ protein, tau phosphorylation and inflammatory cytokine levels (IL-1β and TNF-α) was investigated. It was found that minocycline significantly decreased tau hyperphosphorylation and Aβ production. Minocycline may also reduce the self-perpetuating cycle between neuroinflammation and the formation of Aβ and protein tau aggregates. However, the results obtained by immunostaining did not demonstrate this effect.

In a study recently performed by Medina et al. (2021) doxycycline was proposed as a potential candidate for drug repurposing in AD and other tauopathies due to its ability to disrupt tau aggregation in both heparin-induced systems and K18 self-assembling heparin-free systems. The authors found that in a cell free system doxycycline prevented tau seeding whereas in cell cultures the drug was able to reduce the toxicity of tau aggregates. These tau species exhibited less hydrophobic surfaces and contained lower levels of β-structures.

Costa et al. (2011) studied the effect of doxycycline in a *Drosophila melanogaster* model of AD by measuring life span and climbing ability. It was found that the drug had no effect on the longevity of the Aβ42-expressing flies but improved the locomotor deficit (climbing ability) being also able to partially rescue the rough eye phenotype induced by expression of the Arctic Aβ42 peptide. In the study, when measuring aggregation of Aβ42 peptide and size of the generated species in the presence or absence of doxycycline by means of thioflavin T binding assay and DLS, the ability of the drug in decreasing the formation of amyloid aggregates, the number of fibrils, and the size of Aβ species was demonstrated.

Garcez et al. (2017) investigated the effect of minocycline on spatial memory, neurotrophins and neuroinflammation in a mouse model of AD induced by the administration of Aβ (1-42) oligomer. The radial arm-maze test was used for measuring the effect of the drug on memory outcomes. Minocycline reversed the memory impairment caused by Aβ (1-42) resulting in improved spatial memory. Moreover, *in vitro* tests showed that the drug reduced in the hippocampus the increased levels of IL-1β, IL-10, TNF-α, and brain-derived neurotrophic factor (BDNF) caused by Aβ (1-42). In the cortex minocycline also reduced the high levels of IL-1β, IL-4, TNF-α, and nerve growth factor (NGF).

To determine whether inhibition of microglial activation protects hippocampal neurogenesis and improves cognitive deficits, a study was designed and conducted by Biscaro et al. (2012), in which minocycline was tested as a regulator of microglia activity in a transgenic mouse model of AD. The results obtained showed that in the presence of Aβ pathology, modulation of microglial function with minocycline can protect hippocampal neurogenesis. Interestingly it was observed that upregulation of anti-inflammatory factors and downregulation of pro-inflammatory cytokines as well as improved recognition memory, were associated with the administration of minocycline to transgenic AD mice. These results confirmed the involvement of microglia in the inhibition of adult neurogenesis during brain β-amyloidosis.

In a recent double-blind randomized clinical trial conducted by Howard et al. (2020), the effect of different doses of minocycline was evaluated on cognitive and functional ability in mild AD patients. The study was designed to determine if a 2-year treatment with minocycline could be beneficial. In addition, another objective of this study was to evaluate the dose-dependent effect of the drug in the disease. For this, the drug was tested at two dose levels (200 mg/day and 400 mg/day). Contrary to the expectations, no significant effect on cognitive and functional ability was found when compared with placebo. Another important finding was that the number of patients who completed the 400 mg treatment period was lower than the lower dose and placebo cohorts, mainly because of the occurrence of gastrointestinal and dermatological side effects. From the results obtained three main limitations of the study were stated; firstly, in mild AD neuroinflammation may not be the main cause of neurodegeneration, but rather a reaction to the pathogenesis of the disease, even though there is strong evidence of neuroinflammation in advanced AD. Secondly, even if neurodegeneration is affected by neuroinflammation, the doses tested were not high enough to exert an anti-inflammatory effect on neurons. For this, the maximum dose of minocycline should be tested which is equivalent to 3–7 g/day. Finally, the anti-inflammatory effect of minocycline on patients may be low and not detected by the clinical trial performed. Not using a biomarker in diagnosing and following the treatment may have impaired the accuracy of the study (Howard et al., 2020).

More recently, two different studies conducted in 2021 (Zhao et al., 2021) and 2022 (Gomez-Murcia et al., 2022) on minocycline and doxycycline, respectively, investigated the effects of these two tetracycline derivatives on cognitive outcomes in AD experimental models. Zhao et al. (2021) found that minocycline was able to reduce Aβ production and hyperphosphorylation of tau protein in the hippocampus. Minocycline mitigated AD-like pathology in APP/PS1 mice by limiting the activation of Cdk5/p25 signalling pathways which resulted in improved cognitive and learning outcomes. The other study demonstrated that doxycycline improved spatial memory abilities in APP/PS1 mice (Gomez-Murcia et al., 2022).

4. Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder after AD and in which motor disabilities occur. In this disease a complex pathological mechanism leads to the destruction of dopaminergic neurons in the substantia nigra (Kalia and Lang, 2015). Oxidative stress, mitochondrial dysfunction, excitotoxicity, NO toxicity and inflammation play also key roles in the development of the disease (Jenner, 2003).

In 2006 two drugs; minocycline and creatine entered phase II clinical trials based on a previous systematic evaluation of potentially disease-modifying compounds for PD. The study was conducted on patients diagnosed with PD in the last 5 years but not yet requiring treatment to manage their symptoms. Both drugs showed better effects than placebo

being subsequently considered for phase III clinical trials (Ravina et al., 2006). Despite the fact that the initial results of this study showed a change in the total Unified Parkinson's Disease Rating Scale (UPDRS) score (Movement Disorder Society Task Force on Rating Scales for Parkinson's Disease, 2003), a more thorough investigation should be conducted as the clinical trial was not designed to evaluate the effect of minocycline in retarding the clinical progression of PD (2003).

In Seidl and Potashkin (2011) published a review on studies performed with various neuroprotective agents. The study, which reviewed articles published between 2001 and 2010, reported that minocycline, creatine, and rasagiline exhibited the most significant neuroprotective effects among known neuroprotectors after conducting cell culture, animal, human, and epidemiological studies. Doxycycline has been also reported to exhibit neuroprotective effects in both MPTP and 6-OHDA-induced animal models of PD (Cho et al., 2009; Lazzarini et al., 2013). This effect was initially thought to be related to its ability to reduce neuroinflammation (Santa-Cecília et al., 2016), but since subsequent studies have shown that anti-inflammatory agents alone are not sufficient to prevent neurodegeneration (Manthripragada et al., 2011), doxycycline is likely to exert its effect through several mechanisms.

Further studies have demonstrated that doxycycline is able to inhibit the fibril formation of amyloidogenic proteins such as Aβ peptide (Costa et al., 2011), PrP peptide (Forloni et al., 2009), and β-macroglobulin (Giorgetti et al., 2011). Additional research conducted by González-Lizárraga et al. (2017) when investigating the potential use of doxycycline in PD examined its effect on α-synuclein as abnormal aggregates of this protein occur in PD. The study showed that the drug transformed α-synuclein oligomers into off-pathway high molecular weight species by altering their structures. Off-pathway species exhibit different β-sheet structural arrangements, ultimately leading to the prevention of fibrillation. The effect of α-synuclein on the destabilization of biological membranes, formation of toxic species and reduction of cell viability is in turn affected by these structural changes. The study suggested that this effect of doxycycline during synergistic function along with other neuroprotective actions of the drug may define new goals for repurposing doxycycline in neurodegenerative disorders. Subsequent research regarding the effects of tetracyclines on α-synuclein was published in 2020 (González-Lizárraga et al., 2020). In this study, the effect of chemically modified tetracycline-3 (CMT-3) was investigated and compared with that of doxycycline and minocycline. CMT-3 is a pharmacologically safe tetracycline derivative with reduced antibiotic effect that can cross the BBB. This study showed that both CMT-3 and doxycycline inhibited α-synuclein amyloid aggregation, but minocycline failed to interfere with this process of α-synuclein amyloid fibril formation. The effect of doxycycline on α-synuclein and its remodeling to off-pathway non-toxic, non-seeding, high molecular weight species, and its effectiveness against synucleinopathies was also confirmed in other studies (Dominguez-Meijide et al., 2021).

The neuropathological picture of CNS-activated glial cells in PD patients is quite like that induced and observed in animal models of PD (Teismann et al., 2003). Different studies have evaluated the neuroprotective effects of tetracycline derivatives especially on microglia showing that both minocycline and doxycycline exert neuroprotective actions by inhibiting microglia activation, with doxycycline being more potent than minocycline due to its higher absorption rate, penetration ability to the CNS, and fewer side effects (Yim et al., 1985; Smith and Leyden, 2005). A study conducted by Santa-Cecília et al. (2016) found that doxycycline might be effective in preventing and retarding the progression of PD and other neurodegenerative diseases in which glial functions are altered. It was shown that doxycycline affected microglia activation by modulating the p38 MAPK and NF-kB signaling pathways. In a dose-dependent manner doxycycline specifically reduced the expression of the IBA-1 microglial marker and the production of ROS, NO and pro-inflammatory cytokines (TNF-α and IL-1b).

Bortolanza et al. (2021) indicated that some tetracycline derivatives could be used to prevent and reduce levodopa-induced dyskinesia in PD patients. In the study, the anti-dyskinetic potential of doxycycline and its analogue compound COL-3 in hemiparkinsonian rats derived from its ability in reducing MMPs-2/-9 activity, MMPs-3 expression and ROS production, responses that showed significant correlation with the intensity of dyskinesia. The study demonstrated that acute administration of doxycycline or COL-3 produced significant anti-dyskinetic effects in rats exhibiting levodopa-induced dyskinesias. Moreover, the co-administration of doxycycline with levodopa prevented the development of axial, limb, and orofacial abnormal involuntary movements (AIMs) in the animals.

5. Multiple sclerosis

Multiple sclerosis (MS) is a chronic demyelinating inflammatory disorder of variable pathologic and clinical features. The clinical phases of MS can be classified as relapsing-remitting multiple sclerosis (RRMS), secondary progressive multiple sclerosis (SPMS), primary progressive multiple sclerosis (PPMS) and, progressive-relapsing multiple sclerosis (PRMS) depending on the disease progression. Present available therapies for MS have very limited effect on preventing the progression of the disease. Moreover, currently approved drugs have two major drawbacks: post-injection site irritation and high treatment costs. However, minocycline could be a therapeutic option due to its high oral bioavailability (95–100%), being also a safe and well tolerated lipophilic compound that easily crosses the BBB (Chauhan et al., 2021).

MMPs are responsible for the degradation of endothelial lining occurring in vessels of MS patients, which facilitates the infiltration of inflammatory cells in the tissue parenchyma. Therefore, they can be used as biological markers for MS providing information about the clinical type, severity, and disability caused by the disease (Benesová et al., 2009).

As mentioned before tetracyclines, especially doxycycline and minocycline have properties beyond their antibiotic features. Due to their immunomodulatory actions, tetracyclines could be effective in neurodegenerative diseases of autoimmune origin (Park et al., 2020). Regarding MS animal studies have demonstrated that tetracyclines exhibit acceptable neuroprotective effects when concomitantly given with the standard MS treatment (Chen et al., 2011). As minocycline and doxycycline act inhibiting MMPs, these compounds could potentially be repositioned as therapeutic agents in the management of MS (Chauhan et al., 2021). Minocycline inhibits MMPs production, specifically MMPs-9, leading to symptomatically improvement of experimentally induced autoimmune encephalomyelitis (EAE) (Brundula et al., 2002). Moreover, the combination of minocycline and hydroxychloroquine has showed a synergistic effect on EAE models which could potentially be interesting in MS, including progressive MS (Faissner et al., 2018).

In a study carried out by Planche et al. (2017) it was found that dentate gyrus vulnerability is the main reason for early memory impairment in MS, with dentate gyrus injury occurring after microglial activation. In the study minocycline was able to prevent this disorder in a mice model of EAE by inhibiting microglia activation. However, further studies should be conducted to clearly demonstrate this potential neuroprotective effect of minocycline.

Chen et al. (2012) showed that minocycline up-regulated the expression of BDNF and NGF factors in peripheral (blood serum and splenocytes culture supernatants) and target organs (lumber spinal cord and cerebral cortex) of both *in vivo* autoimmune encephalomyelitis C57 BL/6 mice and *in vitro* splenocyte culture supernatants. It should be noted that the level of these two neurotrophins are correlated with clinical outcomes occurring in MS. Previous studies have shown that BDNF and NGF levels of patients with full recovery rates from relapsing symptoms were significantly higher than those found in patients with partial recovery rates (Caggiula et al., 2005). Another study performed in an animal model of EAE (Stoop et al., 2012), showed that minocycline could not completely halt the progression of the disorder although it was able to delay its onset also reducing the severity of the symptoms.

The first clinical studies conducted to evaluate the role of tetracycline derivatives (minocycline) in MS patients were published in 2004 and 2007. These pilot studies were initially performed over a period of 6 months (Metz et al., 2004) and continued for 18 months thereafter (Zabad et al., 2007) on patients with active relapsing remitting MS (RRMS). Clinical outcomes and MRI profiles were supported by immunological results demonstrating a positive effect of minocycline on RRMS patients. The results obtained showed that MMPs-9 activity, which is a target of minocycline actions and the mean number of gadolinium-enhanced lesions significantly decreased. Analysis of the open-label minocycline trial carried out for 24 months suggested that the progression of MS could be stabilized with minocycline treatment. In addition, the dosage regimen assayed (100 mg twice daily) was well tolerated by the patients with no reports of withdrawals due to adverse effects.

Another clinical trial carried out by Metz et al. (2009) was designed to improve the performance of glatiramer acetate in the presence of minocycline. In this study, the results of 9-month changes of gadolinium-enhanced lesions on 44 patients with RRMS demonstrated that the risk of relapse tended to be lower in the glatiramer acetate-minocycline combination group versus the glatiramer acetate-placebo group. Afterwards, a study published in 2017 (Metz et al., 2017) examined the effect of minocycline on the conversion of the first demyelinating event, also known as clinically isolated syndrome (CIS), to MS. In this study conducted on 142 CIS patients, the dosage regimen tested (100 mg twice daily) significantly reduced the risk of CIS to MS conversion in the first 6 months but not over the global course of the study period (24 months).

In a double-blind, randomized, placebo-controlled multicentre study (Sørensen et al., 2016) the efficacy and safety of minocycline added to s.c. interferon β-1a therapy was investigated in RRMS patients. The minocycline-treated group received a dose of 100 mg twice daily. The primary endpoint was the time to the first qualifying relapse, and secondary endpoints were the annualized relapse rate for qualifying relapses. In this study, a trend towards a beneficial effect of add-on minocycline was observed for some of the efficacy outcomes. However, minocycline did not show statistically significant beneficial effects when added to s.c. interferon β-1a therapy. It is important to note that due to premature discontinuation of some patients and considering the small number of patients that underwent MRI analysis, this study was not sufficiently powered for evaluating the primary endpoint. Moreover, it must be taken into consideration that both minocycline and interferon β-1a have common advantages in inhibiting MMPs, but the combination of the two immunomodulatory drugs does not necessarily lead to synergistic effects. Minocycline was well tolerated in combination with s.c. interferon β-1a as it was evidenced by the similar rates of side effects described in both groups of patients.

Another clinical trial (Minagar et al., 2008) evaluated the effects of combination therapy based on interferon β-1a and doxycycline in RRMS patients for 4 months. In this open-label trial performed on 15 patients the combination of 100 mg daily oral doxycycline and ongoing i.m. interferon β-1a therapy was found to be effective, safe, and well tolerated, resulting in the reduction of contrast-enhancing lesions and post-treatment Expanded Disability Status Scale (EDSS) values.

A research conducted by Mazdeh and Mobaien (2012) in Iranian patients of MS, doxycycline was used as add-on therapy to interferon β-1a. In this 6-month double-blind study performed in 60 patients no change was observed in favor of the effectiveness of the drug in MRI outcomes, however, the results obtained showed a positive effect of this drug combination in reducing relapses and improving EDSS scores.

However, other studies such as that of Tanaka et al. (2013) expressed some doubts about the positive effects of tetracyclines (minocycline). The findings of this study showed that minocycline was able to inhibit Ciliary Neurotropic Factor (CNTF) in cuprizone-induced demyelination neurons by inhibiting microglia, which finally resulted in reduced remyelination.

6. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is an idiopathic, fatal, and progressive neurodegenerative disorder usually occurring in the fifth decade of life. The disease involves alterations of motor neurons in the brain and spinal cord of unknown origin, thereby resulting in progressive muscle weakness (Kiernan et al., 2011). Genetic findings have linked mutations occurring in various genes to the disease. Missense mutations in ubiquilin 2 (UBQLN2) cause dominant inheritance of ALS with frontotemporal dementia (ALS–FTD) (Le et al., 2016). Currently only two drugs, riluzole and edaravone are approved by the FDA for preventing the progression of ALS (Oskarsson et al., 2018). Moreover, various clinical trials carried out in the last 20 years have not yielded positive results for allowing the introduction of new effective treatments for the disease (Petrov et al., 2017). Due to the presence of the BBB and the blood-spinal cord barrier (BSCB), as well as the properties of the drugs themselves such as low solubility, poor bioavailability and biostability, and off-target effects, traditional drug delivery devices are not able to carry the required doses at the target site. For this, the use of nanotechnology for drug delivery has shown promising results in overcoming biological barriers. It also reduces systemic side effects, increases interaction with the target site, improves bioavailability, and improves biological stability (Wang et al., 2020).

In 2002 three studies (Kriz et al., 2002; Van Den Bosch et al., 2002; Zhu et al., 2002) examined the effects of minocycline on ALS, suggesting that the drug could act by decreasing the activation of p38 MAPK, inhibiting cytochrome *c* release, and down-regulating pro-apoptotic caspase enzymes and pro-inflammatory mediators. They also described minocycline to have a dose-dependent neuroprotective effect. In a follow-up study conducted by Gordon et al. (2004), the use of minocycline in ALS was investigated in a two double-blind, randomized, placebo-controlled study. For this, two trials were designed to study the safety and long-term tolerability of different doses of minocycline in patients with ALS also receiving riluzole. Patients received 200 mg/day of minocycline for 6 months in phase I trial and up to 400 mg/day for 8 months in phase II trial. Overall, a mean dose of 375 mg/day was well tolerated although many patients were able to tolerate higher than standard doses in the phase II trial. Regarding side effects, higher doses of minocycline resulted in gastrointestinal complications and increased blood levels of urea nitrogen. From the results obtained a phase III clinical trial was conducted to study the efficacy of minocycline in a population of 412 ALS patients. In this study, which was performed at an escalating dose of up to 400 mg/day for 9 months, the drug showed similar results than the placebo group. However, clinical deterioration was observed in the minocycline group but not in the placebo group (Gordon et al., 2007).

Gossen and Bujard (1992) developed the so-called tetracycline-induced expression system in which the inducers and inhibitors are tetracycline and its derivatives, especially doxycycline. In this system, the addition of tetracycline or doxycycline to a cell environment was able to induce or inhibit the expression of target genes (Kikuchi and Yoshida, 1983). Genetic studies have linked abnormal protein accumulations in both ALS and frontotemporal lobar degeneration (FTLD) conducting to mutations in UBQLN2 (Teyssou et al., 2017). Selectively expressing mutant $UBQLN2^{P497H}$ in the spinal motor neurons caused progressive degeneration of motor neurons, denervation of skeletal muscles, and abnormal protein accumulation, which are prominent features of ALS. The results of genetic studies have shown that selectively expressing mutant $UBQLN2^{P497H}$ in motor neurons is sufficient to develop ALS in rats.

Akhtar et al. (2018) used a combination of gene therapy and cell therapy with a doxycycline-regulated vector to induce and reverse the expression of glial cell line-derived neurotrophic factor (GDNF) in stem cells. Transplantation of these neurons into the brains of adult mice and following the process of GDNF secretion showed that doxycycline administration could induce and reversibly modulate the *in vivo*

secretion of GDNF.

In addition to motor neurons, glial cells including astrocytes and microglia, are also involved in the pathogenesis of ALS (McGeer and McGeer, 2005). Activated microglia can function as both pro-inflammatory (M1) and anti-inflammatory (M2) factors. M1 microglia expresses pro-inflammatory molecules including TNF-α, IL-1β, interferon-γ (IFN-γ), and NO as well as cell surface markers, CD86 and CD68 (Kigerl et al., 2009). Minocycline selectively acted preventing microglial polarization to M1 without producing effects on M2 (Kobayashi et al., 2013).

A research conducted by Pinkernelle et al. (2013) in organotypic rat spinal cord cultures, concluded that long-term use of tetracycline impaired motor neuronal survival and glial function. In this study, administration of minocycline at dose levels of 10 and 100 μM failed to show satisfactory neuroprotective effects, being the adverse effects justified by inhibiting the activation of both types of microglia during the acute phase of the injury.

Other studies conducted in mice have shown that early asymptomatic administration of minocycline can delay the onset of the disease by inhibiting inflammation and glial activation (Zhang et al., 2003). However, administration of the drug once the disease is diagnosed has not yet yielded positive efficacy results (Gordon et al., 2007; Keller et al., 2011).

7. Advances in neuroprotection based on tetracycline (doxycycline, minocycline) nanosystems

In the past few decades biodegradable controlled release nanocarriers such as polymer-based nanoparticles, liposomes, micelles, solid lipid nanoparticles, dendrimers, and nanoemulsions have received much attention for the efficient delivery of several therapeutic agents to the CNS when targeting neurodegenerative disorders, as they exhibit several potential advantages such as site specificity delivery, improved effectiveness, and prolonged duration of action, among others (Naqvi et al., 2020).

Regarding the potential use of minocycline/doxycicline-based nanosystems in neurodegenerative diseases, Table 1 summarizes the studies found in the literature.

Papa et al. (2013) evaluated minocycline-loaded NPs composed by a polymer based on poly-ε-caprolactone (PLC) and polyethylene glycol when modulating activated microglia/macrophages and the associated proinflammatory events occurring in SCI. Minocycline was selected due to its anti-inflammatory effect on preventing pro-inflammatory mediators such as cytokines IL-1β, IL-6, and TNFα. Interestingly, PLC-based NPs were captured by activated microglia cells within 24–48 h whereas NPs were not entrapped by unstimulated cells. *In vitro* and *in vivo* tests showed that treatment with minocycline PLC-based NPs reduced the activity and proliferation of microglia/macrophages at the site of injury as estimated by decreased expression of pro-inflammatory cytokines IL-6 and CD68. In addition, a decrease in cells with phagocytic phenotype was observed in favour of resting-like phenotype cells. The authors stated that the nanocarrier showed a significant advantage over traditional methods of treating inflammation with anti-inflammatory drugs by achieving improved therapeutic effects, good biocompatibility, and fewer side effects.

Nagpal et al. (2013a) developed different delivery systems for minocycline hydrochloride using chitosan as biocompatible and biodegradable polymer; minocycline-loaded NPs (MHNP) and minocycline-loaded NPs coated with Tween 80® (cMHNP). The formulationswere evaluated for enhanced brain uptake by *in vivo* biodistribution studies carried out in male Wistar rats. Pharmacodynamic studies were also performed in male Swiss albino mice. Moreover,

Table 1
Neuroprotective studies of tetracycline derivatives (doxycycline and minocycline) formulated in nanosystems. PLC (Poly-ε-Caprolactone); NPs (Nanoparticles); SCI (Spinal Cord Injury); GABA (Gamma-Aminobutyric Acid); GSH (Glutathione); PAMAM (Poly(Amidoamine); Dox (Doxycycline); LPS (Lipopolysaccharide); PLGA (Poly D,L-Lactic-co-Glycolic Acid).

Research object (compound)	Type of study	Effect	Refs.
Minocycline PLC-based NPs	SCI model, mice	– Reduced activation and proliferation of microglia/macrophages around the lesion site	Papa et al. (2013)
Minocycline hydrochloride (MH) and encapsulated within chitosan-based MH-loaded NPs coated with Tween 80 (cMHNP)	Biodistribution study: Wistar rats, healthy Pharmacodynamic study, Swiss male albino mice	– Enhanced brain uptake of cMHNP vs MH – Enhanced learning and memory activities – Nootropic activity	Nagpal et al. (2013a)
Minocycline hydrochloride (MH) and encapsulated within chitosan-based MH-loaded NPs coated with Tween 80® (cMHNP)	Behavioural study and evaluation of antidepressant-like activity, Swiss male albino mice Safety study, Wistar rats	– Better tolerance – Better profile of cMHNP vs MH – Improved antidepressant like effect	Nagpal et al. (2013b)
Doxycycline hydrochloride encapsulated within chitosan-based Tween 80® -coated NPs (DCNPopt)	Ketamine-induced psychosis, Swiss albino male mice,	– Increased GABA and GSH levels and decreased TNF-α – Decreased immobility duration	Yadav et al. (2017)
Albumin coupled, chitosan stabilized minocycline+methyl prednisolone-loaded NPs	SCI model, rats	– Decreased lesion volume, improved functional outcomes – Decreased TNF-α, IL-1β, IL-10 levels	Bin et al. (2017)
Hydroxyl-generation-6 (G6) poly(amidoamine) PAMAM dendrimer–9-amino-minocycline conjugate (D-mino)	Cerebral injury, rats *In vitro*: BV-2 cells	– Inhibition of NO release from activated microglia – Inhibition of LPS-induced TNF-α production	Sharma et al. (2017)
Neurotensin (NTS)-polyplex NPs containing a Dox-regulated plasmid	PD model, Wistar rats	– Activation of hGDNF expression in transfected dopaminergic neurons	Espadas-Alvarez et al. (2017)
Polysialic-acid-based minocycline-loaded micelles (PSM)	SCI model, rats *In vitro*: BV2 cells, SH-SY5Y cells, primary microglia	– Protection of neurons and myelin sheaths from damage – Recruitment of endogenous neural stem cells to the lesion site – Reduced glial scar formation – Extension of long axons throughout the glial scar – Promotion of neuron regeneration – Improved locomotor function	Wang et al. (2019)
Minocycline-loaded PLGA NPs (MC-NPs)	Brain implantation, transgenic mice	– Attenuated activation of microglia around the implantation site	Holmkvist et al. (2020)

biochemical tests evaluated their antioxidant and nootropic properties. All formulations, including minocycline hydrochloride in solution, showed significant antioxidant activities due to decreased lipid peroxidation, increased catalase and reduced glutathione (GSH) levels in the brain of mice. Formulation cMHNP demonstrated improved antioxidant and pharmacodynamic activities, reduced brain nitrite levels, and enhanced learning and memory capacities which were related to improved brain uptake of minocycline from this formulation.

These authors (Nagpal et al., 2013b) also studied the safety and efficacy of brain targeted cMHNP NPs compared to the administration of minocycline hydrochloride. For this, male Wistar rats were given increasing oral doses of free drug and formulations MHNP and cMHNP. Rats were observed for 28 days for body weight, behavioural, biochemical, and histopathological changes. The maximum tolerated dose was found to be 350 mg/kg for formulation cMHNP and 319 mg/kg for minocycline hydrochloride. Neither significant side effects nor mortality were reported at the doses tested. Encapsulation of the drug within Tween 80®-coated NPs (formulation cMHNP) significantly improved the antidepressant-like effects of minocycline regarding the duration of the immobility period using the despair swim test (DST) and the tail suspension test (TST) in Swiss male albino mice. In this case the dose tested was equivalent to a 100 mg/kg dose of the free drug. On the other hand, no significant effect was observed for the MHNP treated group. This study demonstrated that surface modified NPs (cMHNP) increased the neuroprotective effects of minocycline also resulting in a better tolerance profile for the drug.

Tween 80® coated chitosan-based NPs were also developed by Yadav et al. (2017). In this study, doxycycline hydrochloride was selected as neuroprotective agent to evaluate its effects on ketamine induced psychosis in Swiss albino male mice. The optimized nanoparticulate system (DCNPopt) was compared to the administration of the free drug at a dose equivalent to 25 mg/kg of doxycycline hydrochloride. The results obtained showed that the nanosystem easily crossed the BBB exerting significant antipsychotic activity. Moreover, oral administration of formulation DCNPopt significantly enhanced GABA (Gamma-Aminobutyric Acid) and GSH levels and diminished malondialdehyde (MDA), TNF-α and dopamine levels at half the dose of free doxycycline hydrochloride,

In Bin et al. (2017) compared the effect of an albumin coupled, chitosan stabilized nanoparticulate system over a non-targeted drug carrier in the treatment of hemisectioned spinal cord injury (SCI). Interestingly, targeted delivery of minocycline-and methyl prednisolone-loaded NPs exhibited a controlled release pattern showing improved therapeutic efficacy as compared to non-targeted NPs. Administration of the targeted NPs to rats resulted in a significant reduction of lesion volumes and improvement of behavioral outcomes. Regarding anti-inflammatory efficacy, targeted NPs led to lower cytokine levels when studied in LPS-induced inflammation in astrocytes. In addition, the non-toxic effect of minocycline was demonstrated in cell viability tests. In the study both drugs (methyl prednisolone and minocycline) showed anti-inflammatory and neuroprotective effects even at a dose one tenth that often used for systemic therapy. The effect was attributed to the site specific and controlled release of the drugs at the inflammatory site.

Sharma et al. (2017) investigated the *in vitro* efficacy and *in vivo* targeting ability of hydroxyl-generation-6 (G6) poly(amidoamine) PAMAM dendrimer–9-amino-minocycline conjugate (D-mino). In this study, researchers used the unique physicochemical features of dendrimers and the anti-inflammatory effect of minocycline to optimize drug delivery to inflammation sites in the brain. *In vivo* results revealed that the D-mino conjugate enhanced the intracellular availability of the drug as the dendrimer conjugate facilitated its passage across the BBB thereby reaching the site of injury. The D-mino conjugate was also able to suppress microglia-mediated inflammation because of its cell-specific delivery to activated microglial cells. The *in vitro* study conducted in LPS-activated murine BV-2 microglial cells demonstrated that D-mino markedly reduced oxidative stress by suppressing NO production and inflammatory cytokine TNF-α production.

In Espadas-Alvarez et al. (2017) developed Neurotensin (NTS)-polyplex NPs containing a single bifunctional doxycycline-regulated plasmid in order to obtain an adequate alternative for viral vectors and destined to regulate human GDNF (hGDNF) gene expression. The formulation was assayed in a PD model induced in male Wistar rats. The results showed that NTS-polyplex NPs containing the doxycycline-regulated plasmid were able to maintain transfection specificity resulting in up-regulation of transgene expression, as hGDNF expression occurred in DA neurons. Moreover, this expression was switched on by doxycycline administration and switched off by its withdrawal. The authors therefore proposed this specific doxycycline-regulated system as suitable for nanomedicine-based treatment of PD.

When using viral vectors the risk of tumorigenic and immunogenic consequences cannot be ruled out (Deyle and Russell, 2009). However, NTS-polyplex NPs did not exert these effects when used in gene therapy with hGDNF. Moreover, they did not cause local or systemic inflammation. Authors claimed that the doxycycline-regulated transgene expression system assayed at the regulatory doxycycline doses reported herein (5 mg/kg) were safe without producing transcriptional “leakage” in the absence of doxycycline.

A recent study conducted by Wang et al. (2019) examined the effect of polysialic-acid-based minocycline-loaded micelles (PSM) on neuronal regeneration and remyelination in a rat SCI model. The researchers claimed that PSM could be a novel strategy in the treatment of other CNS diseases due to their synergistic effects when compared to free minocycline hydrochloride. PSM resulted in significant anti-inflammatory and neuroprotective activities both *in vitro* (BV2 cells, SH-SY5Y cells and primary microglia) and *in vivo*. The administration of PSM significantly safeguarded neurons and myelin sheaths from damage, recruiting endogenous neural stem cells to the lesion site, reducing the formation of glial scars, and promoting the regeneration of neurons and the extension of long axons throughout the glial scars. Regarding the improvement of locomotor symptoms, PSM showed the best therapeutic results with the highest score in the Basso-Beattie-Bresnahan locomotor rating scale when compared to free minocycline hydrochloride.

Holmkvist et al. (2020) have recently demonstrated that local delivery of minocycline-loaded PLGA NPs (MC-NPs) was able to attenuate acute tissue responses nearby brain implantation sites in transgenic mice. Immunohistochemical analyses of brain tissues showed that MC-NPs induced significant activation of microglial cells (CD68-positive cells) without effecting the overall population of microglial cells around the implantation sites. No toxic effects were observed in the study group during the 7-day trial period.

8. Discussion

Neurodegenerative diseases continue to be a global challenge in research for effective treatments. For instance, despite the intensive preclinical and clinical studies performed in PD and the positive results reported in the literature, there is still a lack of effective treatments for the disease. One of the main drawbacks of using experimental animal models is that they only allow studying part of the pathological mechanisms involved in PD. In this regard some researchers suggest that using a combination of neuroprotective agents and/or new drug delivery systems may be a promising strategy to address these issues (Salamon et al., 2020).

In clinical studies, the separation of early symptomatic benefit from disease-modifying effects is a complex task and the corrections made, such as extending the washout period and extending the study period, each have their own complications and errors (Kieburtz, 2006). In addition, recognizing the neuroprotective effects of drugs is an important challenge for clinical studies. For instance, the UPDRS score is used in clinical studies carried out in PD patients. However, this score relies

on clinical examinations performed in patients such as evaluation of motor function and performance of daily activities with the assessment of neuroprotective effects being inaccurate (Movement Disorder Society Task Force on Rating Scales for Parkinson's Disease, 2003).

A valid biomarker could be much more accurate in confirming the diagnosis or objectively measuring the progression of the disease and the effectiveness of the pharmacological treatments assayed. Moreover, because such a biomarker has not been identified to date, it can be stated that clinical outcomes are not necessarily consistent with neuronal changes occurring in PD patients and other neurodegenerative diseases.

The same situation applies to clinical studies performed for AD and ALS. Despite the positive results obtained for tetracyclines in experimental animal models of both diseases, clinical studies still do not show any significant effects when compared to placebo. Biomarkers could therefore play key roles as diagnostic tools in neurodegenerative disorders.

Nanosystems represent a significant breakthrough in drug delivery due to their potential to target a particular organ or tissue, either passively or actively. Enhanced solubility and dissolution rates, improved oral bioavailability, reduced side effects and reduced frequency of administrations, are some of the advantages of nanosystems. Moreover, nanosystems can be formulated to effectively carry drugs across the BBB when targeting the CNS to treat neurodegenerative diseases, among others.

Skidmore et al. (2003) demonstrated that daily administration of doxycycline (20–40 mg) maintained anti-inflammatory effects without causing any bacterial susceptibility. In most CNS models the dose of minocycline required to achieve neuroprotective effects ranges between 20 and 100 mg/kg, being significantly higher than that used in inflammatory and infectious diseases (around 3 mg/kg/day) (Zhang et al., 2015). On the other hand, high doses of minocycline can exacerbate neurotoxicity (Yang et al., 2003). Therefore, targeted drug delivery can reduce the required dose by improving bioavailability and therapeutic efficacy (Sharma et al., 2017). According to these findings, if tetracyclines such as minocycline and doxycycline were formulated in nanosystems, due to improved cell targeting and bioavailability, the desired therapeutic effect could be achieved with the administration of lower doses and the risk of side effects could be reduced.

Kashi et al. (2012) obtained minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) with minocycline-loaded NPs which were reduced by two-fold when compared to free minocycline. Moreover, a synergistic antibacterial activity was demonstrated for electrospun doxycycline-loaded hybrid nanofibrous scaffolds composed by hydroxyapatite nanoparticles (nHA), PCL and gelatin polymers. Co-delivery of nHA particles and doxycycline resulted in inhibition of bacterial growth more efficiently than the delivery of either doxycycline or nHA alone at the same concentrations (Ramírez-Agudelo et al., 2018).

When developing nanosystems for specific therapeutic purposes the choice of polymer and type of formulation can be as important as that of the active ingredient. It is of critical importance to achieve adequate release of the drug from the device, specific delivery to the target site and to reduce toxicity. For instance, in a study performed by Bin et al. (2017) the use of booth chitosan and PLGA as drug-carrying polymers largely determined the controlled release of minocycline and methyl prednisolone and their effective delivery to the site of injury. Minocycline+methyl prednisolone-loaded NPs showed improved therapeutic efficacy as compared to non-targeted NPs. Degradation of the outer chitosan layer followed by erosion of the PLGA matrix ensured controlled drug delivery to the target site, thereby resulting in lower cytotoxicity.

However, despite the potential of nanomedicines, up to date just a few drug products such as Genexol-PM®, Abraxane®, Kadcyla® have entered the pharmaceutical market by receiving FDA approval. These new medications have shown to have significant efficacy effects when compared to their conventional dosage forms (Li et al., 2017). Therefore, the field of nanomedicine is receiving great attention for the development of new drug delivery systems aimed to treat several disorders including neurodegenerative diseases.

9. Conclusion

Tetracyclines have proven to exhibit good neuroprotective effects at the cellular level with different mechanisms involved, such as inhibition of microglial activation, reduction of apoptosis, and inhibition of ROS production. Animal studies have also demonstrated that tetracyclines can alter atrophy by increasing the expression of muscle specific regulatory factors, which are essential for myogenesis thereby resulting in muscle recovery (Shefer et al., 2008), but still have not proven effectiveness in human clinical studies.

Minocycline and doxycycline have been intensively investigated both *in vitro* and *in vivo* for their potential uses in neurodegenerative diseases due to both anti-inflammatory and neuroprotective effects. Moreover, to improve drug arrival at the target site and reduce peripheral side effects the use of nanotechnology is a promising approach which can also facilitate the passage of drugs across the BBB when targeting the CNS. Several nanosystems have been developed and investigated for minocycline and doxycycline for their potential use in neurodegenerative diseases. However, further research is still needed to explore their effectiveness.

CRediT authorship contribution statement

Mahdieh Rahmani: Investigation, Methodology, Validation, Visualization, Writing – original draft. **Sofía Elisa Negro Álvarez:** Conceptualization, Supervision, Writing – review & editing. **Emilia Barcia Hernández:** Conceptualization, Supervision, Writing – review & editing.

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