Tetracyclines: Drugs with Huge Therapeutic Potential

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Abstract: Tetracyclines are an amazing class of chemical agents with multiple therapeutic potential. Structural modification of the original natural tetracyclines led to the synthesis and development of doxycycline and minocycline, compounds with higher lipophilicity, better oral pharmacokinetics and higher potency. Due to diverse pharmacological properties, these drugs are now under extensive investigation for use in the treatment of various disparate diseases. In recent years, several studies have conclusively reported anti-inflammatory, immune-modulating and neuroprotective effects of these compounds. There are currently over 200 ongoing clinical trials on tetracyclines. These studies extend over a wide range of diseases including dermatological diseases, behavior and mental disorders, immune system disorders, cardiovascular diseases, and cancer. In this review we will discuss the chemistry and pharmacology of these agents, and describe how their inhibitory effect on matrix metalloproteinase and on pro-inflammatory cytokines has kindled renewed interest in them. Based on the reports from pre-clinical and clinical trials, the therapeutic potential and application of tetracyclines may well be redefined and extensively extended.

Keywords: Tetracyclines, minocycline, doxycycline, antibiotic, inflammation, neurodegenerative diseases, stroke, matrix metalloproteinase, cytokines, apoptosis.

INTRODUCTION

Tetracyclines are a group of antibiotics with diverse biological activity. These drugs can chemically recognize and bind to many proteins, supramolecular assemblies, and receptors in both bacterial and mammalian cells. Once receptor-bound, tetracyclines modulate various biological processes, homeostatic mechanisms, and cellular pathways, and thereby affect disease pathogenesis. While tetracyclines have been typically used as antibiotic agents against a broad spectrum of bacteria as protein synthesis inhibitors, they have now found novel non-antibiotic usage on cellular mechanisms unrelated to ribosomal protein synthesis [1]. Suppression of matrix metalloproteinase (MMPs) and pro-inflammatory cytokines plus inhibition of apoptosis are examples of such diverse yet highly beneficial effects.

History

Tetracyclines were first discovered in 1948 by Benjamin Duggar [2] as natural compounds, produced by species of Streptomyces, and have since proven to be an effective and economically valuable class of drugs. Streptomyces is a genus of soil-dwelling bacteria that produce chemically diverse natural products, including tetracyclines. As a result, tetracyclines can be easily and cost effectively isolated by fermentation, which has contributed to their extensive use in human therapy, veterinary medicine, animal growth promotion, and aquaculture [3, 4]. Chemical isolation and purification led to discovery of chlortetracycline, the first tetracycline to be fully characterized both chemically and clinically [5]. The compound had a number of advantages over previously discovered penicillins, namely, a larger spectrum of activity (particularly against Gram-negative organisms), and was better-tolerated by patients. Soon after, other natural tetracyclines were isolated, including tetracycline, for which the family of molecules is named. Thereafter, modifications of the naturally occurring tetracyclines and the synthesis of novel compounds within the tetracycline family have generated many compounds. Two of the more common semi-synthetic tetracyclines used clinically as antibiotics are doxycycline and minocycline, which are considered to be highly active and well tolerated [6]. Emergence of drug resistance by certain bacterial strains, led to the development of the glycylcyclines such as tigecycline (9-t-butylglycylamido-minocycline) [7].

ANTI-BACTERIAL ACTIVITY

Despite the development of resistance by some bacterial species, the tetracyclines are still effectively used against both gram-positive and gram-negative bacteria. These compounds are particularly useful in several types of infections, such as atypical pneumonias, community-acquired pneumonia, rickettsial and chlamydial infections, Lyme disease, cholera, syphilis and periodontal infections [8]. Doxycycline and minocycline are the most widely used tetracyclines today. Doxycycline is frequently used as first-line therapy, in the treatment of uncomplicated genital *Chlamydia trachomatis* infections or acute Q fever. Moreover, doxycycline may be used after initial therapy has failed due to decreased antibiotic sensitivity or antibiotic resistance, as in the case of infections with penicillin-resistant *Streptococcus pneumonia* [9]. Minocycline displays broad-spectrum efficacy, as well and is most often used clinically for many of the same infections as doxycycline. One of the most common uses of the tetracyclines and mainly minocycline is in the treatment of acne vulgaris [10].

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It is believed that in acne, the inflammatory reactions profoundly contribute to the pathophysiology of the disease [11]. The tetracyclines and in particular minocycline has been effectively used in the treatment of acne [12-14]. Minocycline is believed to act on several components of the disease including; inhibition of the bacteria (*Propioni-bacterium acnes*), reduced activity of the free fatty acids and the extracellular lipases and suppression of chemo taxis. Moreover, being highly active in the pilosebaceous complex, due to its great lipophilicity, minocycline has been used in the treatment of moderate to severe papulo-pustular acne for a long time [12]. It is believed that in acne, the anti-inflammatory affects are achieved at much lower concentrations than the minimal inhibitory antibacterial concentrations [14]. Minocycline is a useful addition to the range of disease modifying antirheumatic drugs (DMARD) in the management of rheumatoid arthritis (RA), particularly in those with previous major sepsis and are not eligible for anti-tumor necrosis factor therapy. Several randomized controlled trials of minocycline have shown benefit in reducing acute phase response and tender and swollen joint counts [15]. Moreover, by suppressing the expression of HMGB1 (high mobility group box-1, a potent and important mediator in sepsis and inflammatory factor in lung disease) in microglia cells, minocycline has been shown to exert a cerebro-protective effect [16].

MODE OF ACTION

It is well established that tetracyclines traverse the outer membrane of gram-negative enteric bacteria through the OmpF and OmpC porin channels, as positively charged cation (probably magnesium)-tetracycline coordination complexes [3, 17]. The cationic metal ion-antibiotic complex is attracted by the Donnan potential across the outer membrane, leading to accumulation in the periplasm, where the metal ion-tetracycline complex probably dissociates to liberate uncharged tetracycline, a weakly lipophilic molecule able to diffuse through the lipid bilayer regions of the inner cytoplasmic membrane. Similarly, the electroneutral, lipophilic form is assumed to be the species transferred across the cytoplasmic membrane of gram-positive bacteria (Fig. **1**). Uptake of tetracyclines across the cytoplasmic membrane is energy dependent and driven by the ΔpH component of the proton motive force [18]. Moreover, tetracyclines also cause alterations in the cytoplasminc membrane, causing leakage of nucleotides and other compounds from the bacterial cell [19].

At clinically relevant concentrations, tetracyclines are bacteriostatic and inhibit protein synthesis by binding to the 30S ribosomal messenger RNA (Fig. **2**). This prevents binding of aminoacyl transfer RNA to the messenger RNA-ribosome complex [19].

STRUCTURE-ACTIVITY RELATIONSHIP

Tetracycline molecules comprise a linear fused tetra-cyclic nucleus (rings designated A, B, C, and D to which a variety of functional groups are attached at upper and lower peripheral zone (Table **1**).

The simplest tetracycline to display detectable antibacterial activity is 6-deoxy-6-demethyltetracycline and so, this structure may be regarded as the minimum pharmacophore [3]. Features important for antibacterial activity among the tetracyclines are maintenance of the linear fused tetra-cycle, naturally occurring (α) stereochemical configurations at the 4a, 12a (A-B ring junction), and 4 (dimethylamino group) positions, and conservation of the keto-enol system (positions 11, 12, and 12a) in proximity to the phenolic D ring [3]. All tetracyclines must possess a C_4 dimethylamino group for bioactivity.

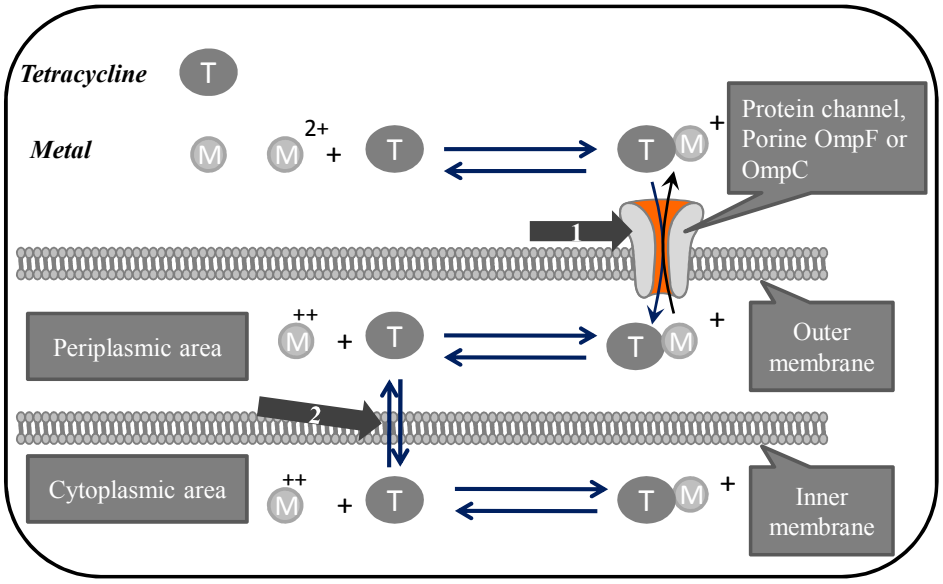


Fig. (1). Uptake and transport of the tetracycline molecule across the Escherichia coli membrane. Tetracyclines pass the outer membrane of gram-negative bacteria through the porins OmpF and OmpC, probably chelating a M^{2+} ion (1). The cationic tetracycline is attracted by the Donnan potential across the outer membrane leading to eventual accumulation in the periplasm, where the tetracycline complex dissociates yielding the uncharged drug molecule. This weakly lipophilic compound is able to diffuse through lipid bilayers and does not depend on a protein channel (2).

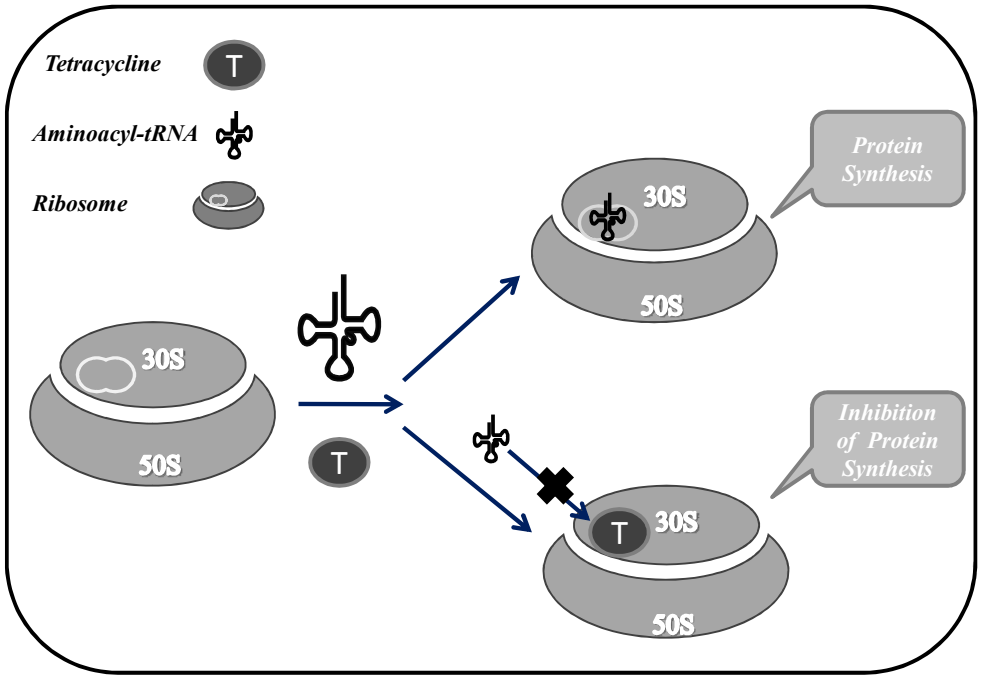
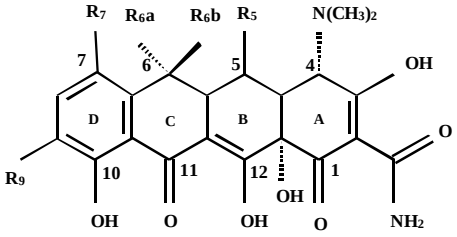


Fig. (2). Tetracyclines exert their antibiotic effect primarily by binding to the bacterial ribosome and halting protein synthesis. Bacterial ribosome has a high-affinity binding site located on the 30S subunit. Upon binding the ribosome, the tetracyclines allosterically inhibit binding of the amino acyl-tRNA at the acceptor site, and protein synthesis ceases.

Table 1. Chemical Structure of Tetracyclines



Compound	R_5	R_{6a}	R_{6b}	R_7	R_9
Tigecycline	H	H	H	H	$NHCOCH_2NHC(CH_3)$
Minocycline	H	H	H	$N(CH_3)_2$	H
Doxycycline	OH	CH_3	H	H	H
Tetracycline	H	CH_3	OH	H	H
Chlorotetracycline	H	CH_3	OH	Cl	H

Synthetic modifications within this region has successfully produced newer generations of antibacterial agents that act by inhibiting protein synthesis at the level of the ribosome [20].

Natural fermentation products or synthetically modified derivatives along the upper peripheral region and on positions 7 through 9 on the D ring of tetracycline has led to the synthesis of molecules with higher activity [20] (Fig. **3**).

The tetracyclines are strong chelating agents [17] and both their antimicrobial and pharmacokinetic properties are influenced by chelation of metal ions. Chelation sites include the beta-diketone system (positions 11 and 12) and the enol (positions 1 and 3) and carboxamide (position 2) groups of the A ring [3]. Most synthetic modifications along the lower peripheral region of tetracycline greatly decrease their biological activity. Tetracyclines bind divalent metal cations, mostly along the lower peripheral region, and circulate in blood plasma primarily as Ca^{2+} and Mg^{2+} chelates. Their role as calcium ionophores has important biologic implications. After intracellular incorporation, Ca^{2+} can act as a secondary messenger and affect pathways such as secretary processes, receptor activation or inhibition, cell division, and metabolic reactions [20]. Moreover, there is a direct relationship

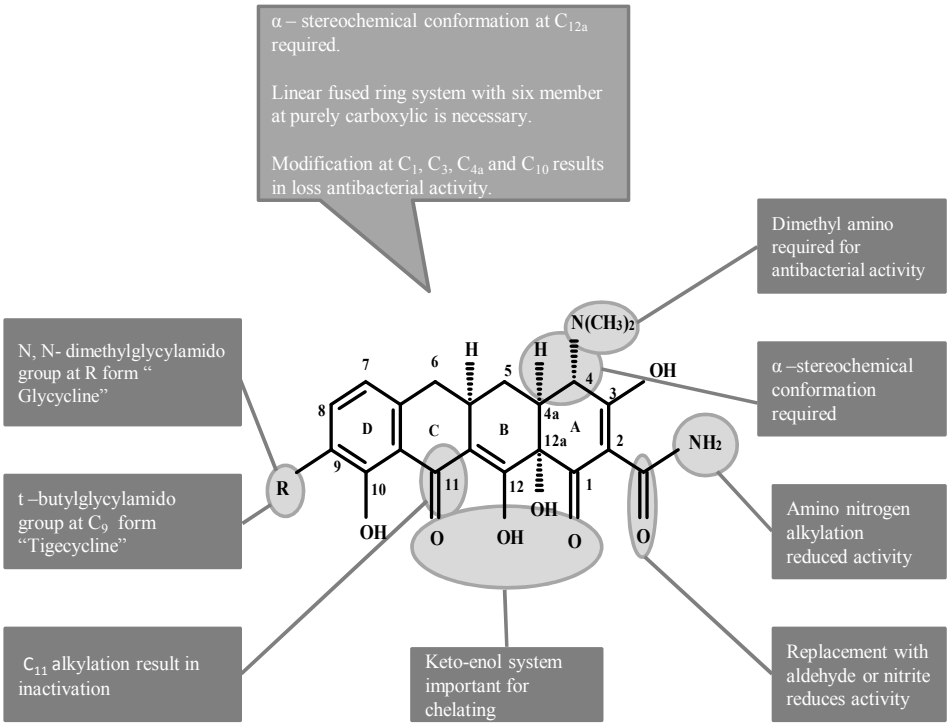


Fig. (3). Structure Activity Relationship.

between lipophilicity and activity against Gram-positive bacteria. Lipophilicity also affects tissue distribution. Minocycline is able to cross the blood-brain barrier much more readily than doxycycline or tetracycline. Minocycline attains levels in the brain nearly threefold higher than doxycycline, while tetracycline is undetectable in the brain [21].

Tetracyclines are not static molecules, changing molecular conformations as pH and other aqueous-phase factors change. An un-ionized form of tetracycline, where intra-molecular hydrogen bonding occurs at the position 3 oxygen, renders the molecule more lipophilic and able to cross lipid bi-layers. Both conformations are believed to be responsible for biological properties *in vivo*, where the unionized form is primarily responsible for membrane permeation and other pharmacokinetic properties, while the zwitterionic form is responsible for affinity-binding to its biological target [20].

RESISTANCE

Eventually, after 4 decades of extensive clinical use, tetracyclines began to decline as first-line antibiotics. This decline was due to the emergence of resistant strains of bacteria. A number of mechanisms have been suggested including; reduction of the intracellular concentration of the compound through active efflux [22], disruption of the tetracycline-ribosomal interaction by ribosomal protection proteins (RPPs) [23], enzymatic inactivation of the drug through mono-hydroxylation [24] and alteration of the target site through 16S RNA mutation [25].

The most common bacteriostatic action of tetracycline is by inactivation of the bacterial ribosome, so that protein biosynthesis is interrupted and the bacteria die. The most common mechanism of resistance in gram-negative bacteria is associated with the membrane protein TetA, which exports the drug out of the bacterial cell before it can attack its target, the ribosome. The expression of TetA is tightly regulated by the homodimeric tetracycline repressor, which binds specifically to operator DNA, upstream of the TetA gene. When tetracycline diffuses into the cell, it is negatively charged, having released a proton, and chelated to form a $tetracycline^{-}$:Mg^{2+} complex. It is the $tetracycline^{-}$: Mg^{2+} complex that binds to tetracycline receptor, inducing conformational changes that sharply reduce the affinity of tetracycline receptor for the DNA. Tetracycline receptor is then released from the DNA and expression of TetA can proceed, conferring resistance on the bacterial cells [26].

STRUCTURE MODIFICATIONS TO OVERCOME RESISTANCE

In order to overcome the resistance, extensive research has led to the development of the glycylcyclines. These compounds have the central four-ring carboxylic skeleton present in the tetracyclines that is necessary for antibacterial activity. In the glycylcyclines, substitution of an N-alkyl-glycylamido group on the D ring at position 9 facilitates the broader spectrum of activity, and additionally creates the ability to overcome most tetracycline resistance mechanisms. Tigecycline a minocycline derivative (Fig. **4**), has a 9-t-butyl-glycylamido side chain on the central skeleton, which is thought to be responsible for steric hindrance and the overcoming of the major resistance mechanisms [7].

In the course of developing compounds with improved activity and pharmacokinetic properties, it was found that, the hydrophilic domain of the tetracycline molecule is important for binding to ribosome and modification at that

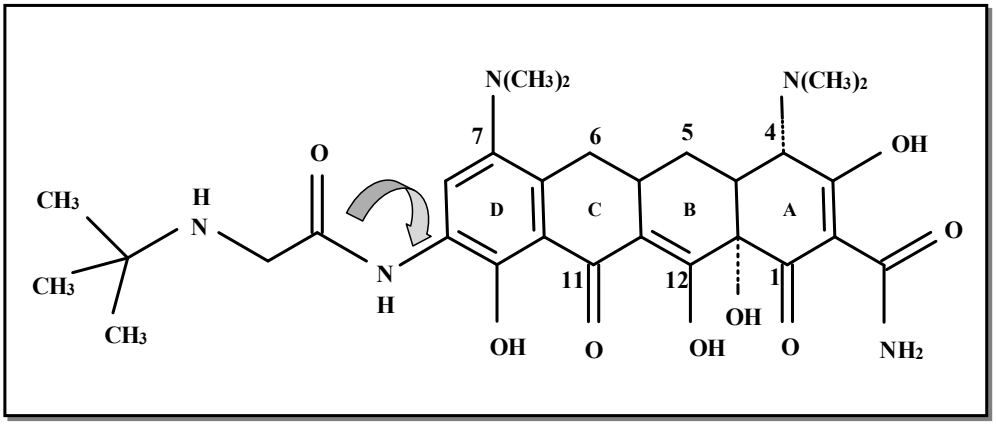


Fig. (4). Structure of Tigecycline. The arrow indicates the addition of a 9-tert-butyl-glycylamido side chain on the D ring at the 9th position.

region would interfere with the binding site, while modification of the hydrophobic region might produce compounds with enhanced antibacterial activity. Additionally it has been reported that, the sulfonamide derivatives are much more active against Gram-positive bacteria than the acylated series. The potent *in vitro* activity of some of the sulfonamide derivatives against resistant Gram-positive bacteria makes them potential candidates for the development of new antibiotics selectively targeting the Gram-positive pathogens [27].

NON-ANTIBIOTIC EFFECTS

In addition to their well-characterized antibiotic effects, extensive research on tetracyclines has unveiled a range of highly valuable pharmacological properties. The non-antibiotic effects are under investigation for the treatment of a wide range of diseases. Most of these potential new indications are in the area of cardiovascular and neurological disorders, involving inflammatory processes arising from disease-induced MMPs, excessive secretion of growth factors, free radicals and condition-induced hyper-secretion of cytokines. In this section we will briefly review the role of these mediators and how by suppressing these, tetracyclines may arrest disease progression.

Inhibition of MMPs

Probably the best characterized non-antimicrobial property of the tetracyclines is their ability to inhibit members of the MMP family of endopeptidases [28]. A family of zinc-dependent proteases, MMPs, have the ability to digest structural proteins of the extracellular matrix (ECM), and thereby play an important role in tissue remodeling associated with various physiological and pathological processes, such as morphogenesis, angiogenesis, tissue repair, arthritis, chronic heart failure, chronic obstructive pulmonary disease, chronic inflammation, and tumor invasion [1, 29, 30].

MMPs identified so far (over 30), are assigned into three major classes of collagenases, gelatinases and stromelysins. While tetracyclines affect a number of the MMP family, their inhibitory effects on the gelatinases (MMP-2 and MMP-9) has been extensively studied and well documented for instance, both MMP-2 and MMP-9 can regulate endothelial permeability and thereby contribute to both inflammation and angiogenesis [28-29]. The broad involvement of these MMPs in a wide range of inflammatory, cardiovascular and malignant diseases means that suppression of synthesis or inhibition of their activity would lead to clinically valuable therapeutic effects [31]. In many instances, and in particular in stroke and cardiovascular diseases, tetracyclines such as doxycycline have been found to be highly effective [32, 33]. An example of this is the implication of MMPs in plaque formation and progression leading to myocardial infarction and stroke. Tetracyclines are effective in reducing plaque formation and disease progression thus leading to improved disease outcome [28-29, 34].

While the therapeutic utility of MMP inhibitors such as doxycycline and minocycline in cardiovascular diseases and stroke are rapidly gaining momentum, the role of MMPs in the progression of cancer still remains open to debate. In brief, MMPs are associated with multiple human cancers and have been targets for anticancer drug development for over 2 decades. However, results from the clinical trials employing the newly developed MMP inhibitors, did not match expectations and were thus abandoned for a few years. Recent developments and progress in our understanding of MMPs have re-kindled the hypothesis of the efficacy of MMP inhibitors in cancer. It is well established that, tumoral invasion and metastases are conditioned through various enzyme activities in particular proteases which degrade the matrix, thus facilitating the progression of the tumor [35]. Cancer cell invasion comprises steps in the destruction of the basement membrane and migration of cells into the connective tissue which through the lymph nodes and vessels migrate to form metastases [36]. An effect largely (but not solely) mediated by the MMPs. Moreover, MMPs are thought to further contribute to tumor cell survival, growth and metastases through activation of tumor growth factor-α and β (TGF-α and TGF-β), epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF), which are potent mediators of both inflammation and cancer [37, 38]. These growth factors inhibit apoptosis, prolong cell survival, and induce cell proliferation, migration and tumor angiogenesis [39]. Thus, inhibition of MMPs by the tetracyclines could contribute to their anti-cancer and in particular anti-metastatic activity in number of ways. Agents targeting these growth factors have been developed, are

under clinical trials or have recently been approved for use in the treatment of cancer. It is therefore rational to benefit from tetracyclines as inhibitors of MMPs in the treatment of cancer.

Following the discovery that, tetracyclines inhibit MMPs by mechanisms which are independent of their antibacterial activity, 4-De-dimethylamino tetracycline or the first chemically modified tetracycline (CMT) was synthesized followed by synthesis and evaluation of many tetracycline derivatives with more selective actions [40]. While CMTs have non-antimicrobial activity due to their inability to adapt a zwitterionic form, they do retain the ability to bind to other non-microbial targets, such as MMPs, thus facilitating their use in the treatment of medical conditions where these proteinases play a vital role in the pathogenesis and progression of the disease [41].

The scenario of using tetracyclines in a wider range of diseases becomes more interesting when one takes into account their regulatory influence on the immune system and inflammation. MMPs have been regarded as major regulators of innate and acquired immunity, playing an important role in both acute as well as chronic inflammation [42]. Increased expression of MMPs has been observed in almost all human diseases where inflammation is present [35]. Depending on the cell type and the disease, MMPs are believed to play a major role in inflammation by regulating physical barriers, modulating inflammatory mediators (cytokines and chemokines), and chemotaxis [43]. Moreover, there is increasing evidence for the tight correlation between MMPs, inflammation and cancer [44].

Anti-Inflammatory Activity

Unregulated, excessive and prolonged inflammation can have devastating effects such as organ failure in short term and numerous debilitating consequences such as cancer in the long term. Tetracyclines moderate inflammatory responses of various etiologies [45]. Anti-inflammatory action of tetracyclines was recognized upon their antibiotic application to several skin diseases. The anti-inflammatory activity of tetracyclines has also been reported in rheumatoid arthritis, corneal inflammation, inflamed gingiva, osteoarthritic cartilage, allergen-induced inflammation, and a number of other conditions [46]. The wide spectrum anti-inflammatory effects of tetracyclines are probably a consequence of their ability to interfere with the synthesis or activity of several mediators of inflammation. In addition to the inhibition of MMPs (collagenases and gelatinases), they are known to suppress hydrolases such as α-amylases and phospholipase A_2 [47]. Phospholipase A_2 is a key enzyme in the biosynthesis of inflammatory mediators such as the prostaglandins [48]. Structure analysis at a high resolution (1.65 Å) clearly shows that minocycline binds to the hydrophobic cleft at the entrance of the active site of phospholipase A_2. As a consequence, the access of substrate molecule to the active site is blocked, and the conformation of the Ca^{2+}-binding loop is stabilized in the Ca^{2+}-free conformation of the apo-enzyme, thus resulting in inhibition of enzymatic activity. Secretory phospholipase A_2 is also inhibited by non-antimicrobial CMTs with lack of dimethylamino group at position 4 of the tetracycline A ring. CMTs might be useful for the therapy of inflammatory processes, since they have no antimicrobial activity but are inhibitors of both pro-inflammatory secretory phospholipase A_2 and some MMPs [47].

Moreover, reactive oxygen species (ROS) generated by white cells are closely correlated with the pathogenesis of a variety of inflammatory and in particular skin disorders [49]. Tetracyclines also scavenge ROS, excessively produced under pathological conditions and thereby prevent or reduce pathological tissue destruction [50].

Another major effect of the tetracyclines is to interfere with cytokine production from immune cells such as neutrophiles and macrophages under inflammatory conditions [51]. This is thought to contribute significantly to the overall anti-inflammatory effect of the tetracyclines and their ability to inhibit cytokine release. Tetracyclines and CMTs suppress cytokine levels such as tumor necrosis factor alpha (TNFα), interleukin 1 beta (IL-1β) and interleukin six (IL-6) under pathological conditions and in particular where inflammation is involved [52, 53]. These cytokines are known to play essential roles in the process of inflammation, hence, antibodies that neutralize the cytokine, their receptors or interfere with their signaling pathways are used in the clinic for the treatment of inflammatory diseases [54-56]. These cytokines also contribute to the neo-angiogenesis in both inflammation and cancer and hence the use of anti-IL-6R antibody and toclizumab [57, 58]. Numerous reports suggest that tetracyclines and in particular minocycline are effective in arresting disease progression in a number of neurodegenerative diseases. The most likely explanation as to how tetracyclines work here, include inhibition of inflammation and microglia activation, inhibition of apoptosis and suppression of ROS production [59]. In brief, IL-6 (and its receptor IL-6R) are found to be expressed by neurons and glial cells in the brain [46] and shown to be dramatically increased in a number of pathological conditions [60]. A similar situation has been identified in heart disease where continuous and excessive production of IL-6 promotes myocardial injury and cardiac hypertrophy [61]. The exact underlying causes are not yet fully understood, but evidence suggests that, the pro-inflammatory cytokines could potentially be highly involved. Enhanced unregulated apoptosis has been recognized as another contributing factor. Numerous studies have shown that tetracyclines inhibit cell apoptosis [62, 63].

Both inflammation and apoptosis have also been linked with several neurodegenerative disorders of the central nervous system (CNS). Inflammatory processes in the CNS are believed to contribute significantly in the pathways leading to neuronal cell death in Parkinson, Alzheimer, Multiple Sclerosis and a range of other neurodegenerative disorders. As an example, MS is an autoimmune/ inflammatory disease of the CNS [36] where the inflammatory response is usually mediated by the activated microglia, the resident immune cells of the CNS [38]. Thus, another major area of interest for the use of tetracyclines and the CMTs in particular is neurodegenerative disorders where inflammation is a contributing factor to disease progression.

Table 2. Ongoing Clinical Trials with Tetracyclines

Research Category	Number of ongoing clinical studies			Most common areas
	Tetracycline	Doxycycline	Minocycline	
Nervous System Diseases	39	50	135	- Central Nervous System Diseases - Brain Diseases - Neurodegenerative Diseases - Autoimmune Diseases of the Nervous System - Multiple Sclerosis
Behavior and Mental Disorders	11	18	37	- Mental Disorders - Psychotic Disorders - Dementia - Cognition Disorders - Schizophrenia and Disorders
Immune System Diseases	27	18	73	-Autoimmune Diseases - Autoimmune Diseases of the Nervous System - Multiple Sclerosis - Demyelinating Autoimmune Diseases, CNS - Acquired Immunodeficiency Syndrome

Other Effects

Extensive pre-clinical research on the effect of the tetracyclines and CMTs on neurological and cardiovascular disorders has led to the recognition of their huge potential in treating a wide spectrum of disorders. Minocycline has been the most widely used tetracycline for conditions requiring adequate drug concentrations within the central nervous system. Being a highly lipophilic drug, minocycline has better penetration through the blood-brain barrier than other tetracyclines [64]. It has been shown to display neuroprotective properties in various animal models of neurodegenerative diseases and to delay motor alterations, inflammation and apoptosis in experimental animal models of Huntington's disease, Amyotrophic Lateral Sclerosis, Parkinson, as well as in stroke, [65, 66].

In line with these protective effects, minocycline has been shown to inhibit microglia proliferation, activation and subsequent release of inflammatory mediators and excitotoxin induced release of nitric oxide, TNFα, IL-1β and IL-6 [67, 68].

Many of the beneficial effects of minocycline in neurological diseases may also be linked to their effects on mitochondria [69, 70], metal ion chelating activity [71] and inhibition of apoptosis [72, 73]. Tetracyclines have multiple effects on mitochondrial functioning. In brief, chelate Ca^{2+} ions and in a Ca^{2+}-dependent manner, bind to mitochondrial membranes, form ion channels in the inner mitochondrial membrane and induce their depolarization [71]. This effect has been linked to inhibition of cytochrome c release from the mitochondria leading to inhibition of apoptosis. Up-regulation of Bcl-2 (a major anti-apoptotic protein), reduced expression and reduced activation of caspases 1 and 3 (inducers and executioners of apoptosis), increased ratio of XIAP to smac/DIABLO (a pro-apoptosis event), reduced mitochondrial release of cytochrome C and smac/DIABLO are some of the described mechanisms accounting for the anti-apoptotic effects of minocycline in the neuron, ultimately preventing neuronal damage [74-76]. These preclinical observations have led to wide spectrum clinical evaluation of tetracyclines and the CMTs (Table **2**).

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