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Tetracyclines and pain

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Received: 23 June 2011 / Accepted: 5 January 2012 / Published online: 27 January 2012
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Abstract Tetracyclines are natural or semi-synthetic bacteriostatic agents which have been used since late 1940s against a wide range of gram-positive and gram-negative bacteria and atypical organisms such as chlamydia, mycoplasmas, rickettsia, and protozoan parasites. After the discovery of the first tetracyclines, a second generation of compounds was

sought in order to improve water solubility for parenteral administration or to enhance bioavailability after oral administration. This approach resulted in the development of doxycycline and minocycline in the 1970s. Doxycycline was included in the World Health Organization Model List of Essential Medicines either as antibacterial or to prevent malaria or to treat patients with this disease. Additional development led to the third generation of tetracyclines, being tigecycline the only medicine of this class to date. Besides antibacterial activities, the anti-inflammatory, antihypernociceptive and neuroprotective activities of tetracyclines began to be widely studied in the late 1990s. Indeed, there has been an increasing interest in investigating the effects induced by minocycline as this liposoluble derivative is known to cross the blood–brain barrier to the greatest extent. Minocycline induces antihypernociceptive effects in a wide range of animal models of nociceptive, inflammatory and neuropathic pain. In this study, we discuss the antihypernociceptive activity of tetracyclines and summarise its underlying cellular and molecular mechanisms.

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Keywords Minocycline · Microglia · Chemically modified tetracyclines · Drug repositioning · Nociception · Inflammation

Abbreviations

BDNF	Brain-derived neurotrophic factor
CCL21	Chemokine (C–C motif) ligand 21
CFA	Complete Freund's adjuvant
CMT	Chemically modified tetracycline
COX	Cyclooxygenase
CX ₃ CL1	Fractalkine
CX ₃ CR1	Fractalkine receptor
GABA	Gamma-amino butyric acid
HIV-1	Human immunodeficiency virus-1

IL	Interleukin
JNK	c-Jun-N-terminal
KCC2	K ⁺ -Cl ⁻ co-transporter 2
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MHCII	Major histocompatibility complex class II
MMP	Matrix metalloproteinase
NFAT1	Nuclear factor of activated T-cells 1
NF-κB	Nuclear factor-kappa B
NGF	Nerve growth factor
NMDA	N-Methyl-D-aspartate
PARP-1	Poly(ADP-ribose) polymerase-1
PG	Prostaglandin
PKC	Protein kinase C
PLA ₂	Phospholipase A ₂
PMIN	12S-Hydroxy-1,12-pyrazolinomincycline
TLR4	Toll-like receptor-4
TNF-α	Tumour-necrosis factor-alpha
WHO	World Health Organization

Introduction

According to the pharmacologist and Nobel Prize winner James Black, “the most fruitful basis for the discovery of a new drug is to start with an old drug”. Indeed, the number of approved new chemical entity medicines has been decreasing (Mullard 2011) as the number of drugs repositioned is increasing (Ashburn and Thor 2004; Tobinick 2009). Therefore, identifying and developing new uses for existing drugs appears to be more rational and promising than searching for new chemical entities, especially because pharmacokinetic and toxicological data are already available. Recently approved new chemical entity medicines are considered potentially unsafe until post-market surveillance studies are conducted. Furthermore, the current legal regulation of the protection of intellectual property is a big challenge worldwide. The strategy of drug repositioning also allows covering the diseases neglected by big pharmaceutical companies.

An example of successful drug repositioning is the non-antibacterial use of the antibiotic doxycycline (Table 1), a second-generation tetracycline. The use of sub-antibacterial doses of doxycycline is approved in the United States of America for the treatment of patients with moderate acne (Skidmore et al. 2003) and worldwide in the prophylaxis and treatment of malaria. This medicine is considered cost-effective and safe for these antimalarial uses by the World Health Organization (WHO), thus justifying its inclusion in the WHO Model List of Essential Medicines (WHO 2009). Since malaria is a neglected disease and still unsatisfactorily prevented and treated, old drugs have been screened to identify more efficacious and safer antimalarial agents (Chong et al. 2006).

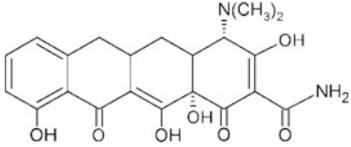
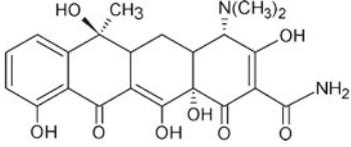
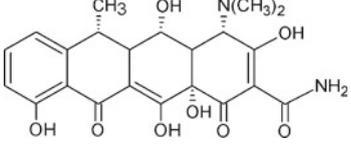
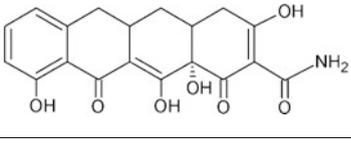
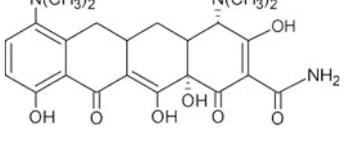
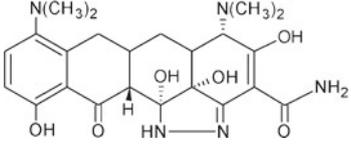
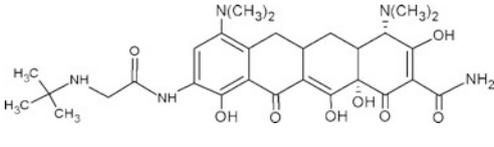
Not only is the antibacterial spectrum of activities exhibited by tetracyclines broad, but the non-antibacterial spectrum of activities is as well. Tetracyclines exhibit pleiotropic activities unrelated to their antibacterial effects, including anti-inflammatory, antihypernociceptive and neuroprotective ones. Experimental studies have shown beneficial effects induced by minocycline (Table 1) in animal models of focal (Yrjanheikki et al. 1999) or global (Yrjanheikki et al. 1998) cerebral ischemia, Alzheimer's (Seabrook et al. 2006), Huntington's (Chen et al. 2000) and Parkinson's (Du et al. 2001; Wu et al. 2002) diseases, amyotrophic lateral sclerosis (Zhu et al. 2002), multiple sclerosis (Brundula et al. 2002) and nociceptive, inflammatory and neuropathic pain (Raghavendra et al. 2003; Ledebore et al. 2005; Bastos et al. 2007, 2008). These results support the conduction of clinical trials to test the usefulness of minocycline in the treatment of ischemic stroke, Huntington's and Parkinson's diseases, multiple sclerosis, amyotrophic lateral sclerosis, rheumatoid arthritis, and spinal cord injuries (www.clinicaltrials.gov). The antihypernociceptive and neuroprotective activities of tetracyclines have been associated with their well-established inhibition of microglial cell activation. Indeed, microglial cells play a key role in both pain and neurodegeneration (Milligan and Watkins 2009). Herein, we focus on the antihypernociceptive effects induced by tetracycline derivatives rather than the neuroprotective ones. The neuroprotective roles played by minocycline have been recently reviewed (Kim and Suh 2009). As minocycline, a semi-synthetic and second-generation tetracycline, is the derivative which crosses the blood–brain barrier to the greatest extent (Aronson 1980), its antihypernociceptive and neuroprotective activities have been more investigated.

Besides repositioning old drugs, developing new drugs by making slight chemical changes in old drugs is promising. By using this new approach, antihypernociceptive and anti-inflammatory activities exhibited by tetracycline derivatives devoid of antibacterial activity have been investigated in more detail. Since a possible limitation of the prolonged use of tetracyclines would be a negative effect on the microflora or development of antibiotic-resistant microorganisms due to their antibacterial effect, chemically modified tetracyclines (CMTs) devoid of such activity were developed, such as incyclinide (Sandler et al. 2005), 12S-hydroxy-1,12-pyrazolinomincycline and 11,12-pyrazolinomincycline (Lertvorachon et al. 2005).

History and chemistry

Discovered in the 1940s, tetracyclines consist of a family of natural products derived from different species of *Streptomyces* spp. or semi-synthetic compounds. Different from what is expected, tetracycline is not the prototype of this

Table 1 Antibacterial and non-antibacterial tetracycline derivatives

Generic name	Chemical Name	Structure	Status	Features
Non-applicable	6-Deoxy-6-demethyltetracycline		Minimum pharmacophore	Simplest tetracycline to exhibit antibacterial activity
Tetracycline	Tetracycline		Marketed since 1953	First-generation tetracycline Natural compound
Doxycycline	6-Deoxy-5-hydroxytetracycline		Marketed since 1967	Second-generation tetracycline Semisynthetic compound
Incyclinide	4-Dimethylamino sancycline		Undergoing clinical trials	Chemically modified tetracycline derived from doxycycline
Minocycline	7-Dimethylamino-6-demethyl-6-deoxytetracycline		Marketed since 1972	Second-generation tetracycline Semisynthetic compound
Non-applicable	12S-Hydroxy-1,12-pyrazolinomincycline		Demonstration of antioxidant effects in 2005	Chemically modified tetracycline derived from minocycline
Tigecycline	9-(<i>t</i> -Butylglycylamido)-minocycline		Marketed since 2006	Third-generation tetracycline Semisynthetic compound

class because the first natural product to be isolated and marketed was chlortetracycline, a medicine available since 1948. Tetracyclines prevent the binding of aminoacyl-tRNA to the 30S ribosomal subunit, the acceptor site, which stops incorporation of aminoacids and thus inhibits bacterial protein synthesis. These compounds are predominantly bacteriostatic agents with broad spectrum against gram-negative and gram-positive bacteria. Besides their human and veterinarian uses as antibiotics, they have been used in agricultural areas as animal growth promoters in livestock production. The use of tetracyclines in the pharmacotherapy of infectious diseases is limited by some factors, such as the development of more effective antibiotics, the impossibility of use by children younger than 9 years and by pregnant and nursing women.

This absolute contraindication is due to their high affinity to developing teeth and bones, with consequent accumulation in these tissues, possibly causing unsightly cosmetic defects and also functional consequences, such as teeth discoloration, gum dysplasia, dental hypoplasia or bone deformities (Chopra and Roberts 2001).

The first generation of tetracyclines (1948–1963) consists of natural or semi-synthetic compounds characterised by low lipophilicity and poor absorption after oral administration and includes chlortetracycline, tetracycline (Table 1), demeclocycline, methacycline, lincycline and rolitetracycline. Afterwards, a second generation (1965–1972) of compounds with better oral absorption profile, higher lipophilicity, longer half-life of elimination and suitability for intravenous administration

(e.g., doxycycline and minocycline) was developed. Glycylcyclines and aminomethylcyclines belong to the third generation of tetracyclines (1993–present), which have a long side chain at C9 and exhibit broader antibacterial spectrum (Table 1). Tigecycline (Table 1), approved by the U.S. Food and Drug Administration in 2005 and marketed since 2006, is the only medicine from this class developed to date. Amadacycline (or PTK0796, Novartis International AG), an aminomethylcycline, is a congener under development (2009).

Tetracyclines comprise a linear fused tetracycline nucleus, with rings designated A, B, C and D (Fig. 1), to which many functional groups can be attached. The simplest tetracycline to exhibit antibacterial activity is 6-deoxy-6-demethyltetracycline (Table 1) and thus is considered the minimum pharmacophore. As mentioned above, tetracyclines inhibit bacterial protein synthesis by preventing the binding of aminoacyl-tRNA to the 30S ribosomal subunit acceptor site. There is evidence that the antibacterial effect induced by tetracyclines requires the coordination of Mg^{2+} (at bacterial ribosomal site) to the 1,3-ketoenol moiety residing between C11 and C12 at the lower periphery of the molecules (Fig. 1) (White and Cantor 1971). Indeed, tetracyclines chelate other bivalent or trivalent cations such as Ca^{2+} , Fe^{2+} , Zn^{2+} , Bi^{3+} and Al^{3+} . Chelation of cations leads to formation of compounds with reduced intestinal absorption (Poiger and Schlatter 1979).

The first described CMT results from the removal of the dimethylamino group from the C4 position (R1) from the “A” ring of tetracycline hydrochloride, at the upper periphery of the molecule (Golub et al. 1987). The rationale of this pioneering study was the evidence that the dimethylamino group in α -orientation in this position is essential for antibacterial activity (Chopra and Roberts 2001). Among several members of the CMTs, incyclinide (Table 1), also known as CMT-3 or COL-3 (Collagenex Pharmaceuticals, Inc.), seems to be the compound with the best therapeutic potential.

Within the last seven decades, thousands of tetracycline derivatives have been isolated or synthesised, but there has been far less interest in compounds with modifications at the lower periphery (Fig. 1), which would impair interaction with Mg^{2+} and thus abolish the antibacterial activity.

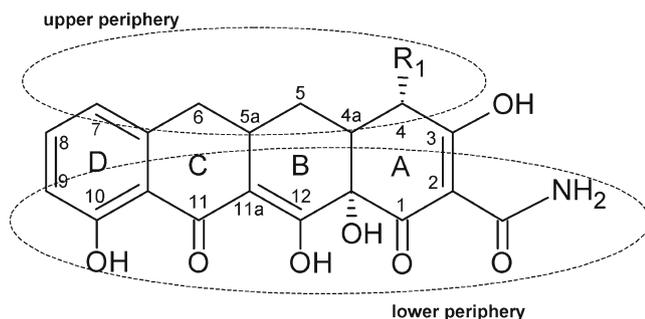


Fig. 1 Linear fused tetracycline nucleus, with designation of rings, carbons and peripheral regions. 181×89 mm (300×300 DPI)

However, in the last decade, Lertvorachon et al. (2005) showed that the reaction of tetracycline, chlortetracycline or minocycline with hydrazine leads to 1,12- and 11,12-substituted tetracyclines, and that one of these products is a potent antioxidant devoid of antibacterial and Zn^{2+} chelating activities. Indeed, 12*S*-hydroxy-1,12-pyrazolinomincycline (PMIN; Table 1) neither inhibits the growth of *Escherichia coli* strains sensitive or resistant to minocycline nor interacts with Ca^{2+} . Afterwards, it was shown that serum from mice treated with a high dose of this compound via intraperitoneal route does not inhibit the growth of a *Staphylococcus aureus* strain. This result indicates that it is unlikely that this compound is converted in vivo into tetracycline derivatives with antibacterial activity (Bastos et al. 2008).

Antihypernociceptive effects

Developing new analgesic medicines from old drugs is worth pursuing because pain management is an unmet health care goal worldwide (Woodcock 2009; Melnikova 2010). Acetylsalicylic acid, ibuprofen and paracetamol are included in the WHO Model List of Essential Medicines in the class of non-steroidal anti-inflammatory medicines, and morphine and codeine are included in the class of opioid analgesics. However, these medicines have limited usefulness for treating patients with painful conditions related to neuropathies (Dray 2008), which were shown to have a prevalence of 7–8% in European countries (Torrance et al. 2006; Bouhassira et al. 2008). Instead of conventional options, some medicines, including gabapentin and pregabalin (antiseizure medicines) and duloxetine (an antidepressant medicine), are approved in some countries for treating patients with neuropathic pain (Dray 2008).

In this context of development of analgesic medicines from old drugs, the antihypernociceptive effects induced by tetracyclines have been widely studied. The effects induced by tetracyclines in models of nociceptive, inflammatory and neuropathic pain were investigated (Table 2), although the effects in nociceptive pain models are not so consistent or replicable. It has been shown that minocycline is inefficient in inhibiting nociceptive behaviour in acute pain models. Neither minocycline nor doxycycline induces an antihypernociceptive effect in the hot plate test (Bastos et al. 2007). Previous treatment of mice with minocycline does not inhibit the writhing response induced by intraperitoneal zymosan or acetic acid (Padi and Kulkarni 2008). In addition, Padi and Kulkarni (2008) showed that minocycline does not alter nociceptive response in the tail immersion test.

Consistent with the observation that minocycline does not inhibit the nociceptive response in experimental models of acute pain, it has been shown that other inhibitors of

Table 2 Major minocycline actions on experimental pain

Model	Species	Effect(s)	Related mechanism(s)	Reference(s)
Formalin test	Mouse/rat	Inhibition of licking or flinching behaviour, second phase is more markedly inhibited	Inhibition of spinal microglial activation and reduction of c-Fos-positive cells	Hua et al. 2005; Cho et al. 2006; Bastos et al. 2007
Hot-plate test	Mouse	No effect	Non-applicable	Bastos et al. 2007
Writhing response induced by acetic acid or zymosan	Mouse	No effect	Non-applicable	Padi and Kulkarni 2008
Intraplantar carrageenan-induced hypernociception	Rat	Antiallodynic/antihyperalgesic	Inhibition of p38 MAPK	Hua et al. 2005; Bastos et al. 2007
Intraplantar IL-1 β -induced hypernociception	Mouse	Antihyperalgesic	Inhibition of microglial activation	Willemen et al. 2010
CFA-induced orofacial, muscular or arthritic experimental pain	Rat	Antiallodynic/antihyperalgesic	Inhibition of microglial activation	Shan et al. 2007; Chacur et al. 2009; Shimizu et al. 2009
Spinal immune activation	Rat	Antiallodynic	Decreased microglial activation, attenuated mRNA expression of IL-1 β , TNF- α , IL-1 β - and TNF- α -converting enzymes, IL-1 receptor antagonist and IL-10 in lumbar dorsal spinal cord, and reduced IL-1 β and TNF- α levels in the CSF	Ledeboer et al. 2005
Spinal LPS-induced hypernociception	Rat	Antihyperalgesic	Decreased spinal PGE ₂ and TNF- α	Saito et al. 2010
Sciatic inflammatory neuropathy	Rat	Antiallodynic	Inhibition of microglial activation	Ledeboer et al. 2005
Spinal nerve ligation	Rat	Antiallodynic/antihyperalgesic	Inhibition of spinal IL-1 β , IL-6 and TNF- α	Raghavendra et al. 2003
Chronic constriction injury	Rat	Antiallodynic/antihyperalgesic	Reduction of serum IL-6 and oxidative stress	Zanjani et al. 2006; Padi and Kulkarni 2008
Streptozotocin-induced diabetic neuropathy	Rat	Antiallodynic	Decreased spinal KCC2 expression	Morgado et al. 2011
Spinal cord injury	Rat	Antiallodynic/antihyperalgesic	Inhibition of spinal p38-MAPK and neuronal excitability	Hains and Waxman 2006; Marchand et al. 2009
Intratracheal CCL21-induced hypernociception	Rat	Antihyperalgesic	Inhibition of microglial activation	Zhao et al. 2007a
Second-degree burn injury	Rat	Antiallodynic	Inhibition of spinal p38-MAPK and neuronal excitability	Chang and Waxman 2010
Cancer-related hypernociception	Rat	Inhibition of allodynia and spontaneous ongoing experimental pain	Inhibition of spinal microglial activation and BDNF production	Wang et al. 2011

macrophage/microglial cell activation, such as pentoxifylline and fluorocitrate, also exhibit this profile (Milligan et al. 2000; Vale et al. 2004). Therefore, these drugs would not inhibit physiological pain, but only pain associated with pathological conditions.

In the formalin test, which exhibits features of nociceptive and inflammatory pain, the results from different studies are seemingly conflicting. After subcutaneous injection of formalin in rodent hind paws, animals immediately start to display a behaviour characterised by flinching, shaking and licking the injected paw. The second phase of this biphasic response is generally inhibited by anti-inflammatory drugs, whereas centrally acting drugs inhibit both phases (Hunskar

and Hole 1987). Hua et al. (2005) showed that intrathecal minocycline reduces the second phase of flinching behaviour induced by formalin in a dose-dependent manner, whereas intraperitoneal minocycline reduces only the first phase in rats. On the other hand, Cho et al. (2006) showed that intraperitoneal minocycline inhibits the second phase of licking behaviour induced by formalin, also in rats. In the same fashion, it was shown that systemic minocycline slightly inhibits the first phase, whereas markedly inhibits the second phase of licking behaviour in mice (Bastos et al. 2007, 2008). Despite differences between species, routes of administration and evaluated behaviours, the results altogether show that inhibition of the second phase is more marked and

reproducible than that of the first one. This suggests that minocycline profile resembles more that of anti-inflammatory drugs, rather than that of analgesic drugs. Doxycycline, besides inhibiting both phases of licking behaviour induced by formalin, inhibits that induced by phorbol 12,13-didecanoate, a phorbol ester known to activate protein kinase C (PKC) (Bastos et al. 2007). Interestingly, PMIN, a non-antibacterial minocycline derivative devoid of Ca^{2+} and Zn^{2+} chelating activities, also inhibits both phases of licking behaviour induced by formalin, and this effect is comparable with that induced by minocycline (Bastos et al. 2008).

Effects induced by tetracyclines in models of inflammatory pain have also been shown and are clearly very consistent and reproducible. Both systemic doxycycline and minocycline inhibit mechanical allodynia induced by intraplantar injection of carrageenan in rats (Bastos et al. 2007). Intrathecal minocycline inhibits thermal hyperalgesia induced by intraplantar carrageenan in rats (Hua et al. 2005) or interleukin (IL)-1 β in mice (Willemen et al. 2010). Intrathecal injection of lipopolysaccharide (LPS), a toll-like receptor 4 (TLR4) agonist, induces mechanical allodynia in a dose-dependent manner, and this nociceptive sensitisation is inhibited by intrathecal minocycline (Saito et al. 2010). Local microinjection of minocycline inhibits thermal hyperalgesia in a model of orofacial pain induced by complete Freund's adjuvant (CFA) injection into the masseter muscle (Shimizu et al. 2009). In a model of muscle inflammatory pain in which CFA is injected into the gastrocnemius–soleus muscle, chronic intrathecal minocycline inhibits mechanical allodynia (Chacur et al. 2009). If CFA is injected directly into the ankle articular cavity of rats, a model of acute monoarthritis, there is development of mechanical allodynia and thermal hyperalgesia, and these phenomena are also inhibited by intrathecal minocycline (Shan et al. 2007).

Minocycline treatment, applied at the time of burn injury in rats and for 1 week thereafter, inhibits microglial activation and induces enduring antiallodynic effect in a protocol that models second-degree burn (Chang and Waxman 2010). Single systemic administration of minocycline induces antiallodynic effect after plantar hind paw incision in rats, a postoperative pain model, if the treatment is applied 24 h after incision, but not 72 h. Even if the late treatment (72 h after incision) is repeated daily up to the seventh day after incision, no antiallodynic effect is observed (Ito et al. 2009).

There is a paucity of therapeutic tools to treat patients with painful conditions of neuropathic origin. Treatments available to date are based on the empirical use of old unconventional drugs (Dray 2008). In this context, minocycline has been undergoing preclinical studies (Raghavendra et al. 2003; Ledebøer et al. 2005; Mei et al. 2011; Morgado et al. 2011). Ledebøer et al. (2005) showed that intrathecal minocycline prevents mechanical allodynia induced by

perisciatic administration of zymosan or that induced by spinal immune activation after intrathecal injection of gp120 from human immunodeficiency virus (HIV)-1. Minocycline treatment inhibits mechanical allodynia in streptozotocin-diabetic rats, a model of peripheral diabetic neuropathy (Morgado et al. 2011). Basing on evidence of neuroprotective effect induced by minocycline and the association of this effect with inhibition of microglial activation (Yrjanheikki et al. 1999), Raghavendra et al. (2003) showed that intraperitoneal minocycline prevents the development of mechanical allodynia and thermal hyperalgesia after L5 spinal nerve ligation. However, no effect on existing mechanical allodynia and thermal hyperalgesia were observed in this study when the treatment was started from day 5 post-ligation up to day 10. More recent data shows that antiallodynic effect can be observed if the treatment (intrathecal injection) is applied 1, 3, 7 days after nerve ligation, but not 10 or 21 days after (Mei et al. 2011). Taken together, these results clearly show that there is short time window for a possible therapeutic intervention. This may be attributable to the fact that the inflammatory process triggers neuroplastic changes which gradually lead to peripheral and central sensitisation related to the development and persistence of allodynia and hyperalgesia (Costigan et al. 2009; Milligan and Watkins 2009; Beggs and Salter 2010). Therefore, once these neuroplastic changes are fully established, only compounds with marked direct inhibitory actions on neuronal hyperexcitability are expected to induce significant antihypernociceptive effects (Dray 2008).

Beneficial effects induced by minocycline in models of spinal cord injury have supported the conduction of clinical trials to test its therapeutic usefulness (Kwon et al. 2010). In this fashion, the antihypernociceptive effects induced by minocycline in these models have been studied (Hains and Waxman 2006; Marchand et al. 2009). Traumatic spinal cord injury induces motor impairment and chronic experimental pain, with concomitant microglial activation. Rats subjected to T9 spinal cord contusion injury display mechanical allodynia and thermal hyperalgesia 4 weeks after injury. Intrathecal minocycline treatment for 3 days, started after sensitisation is established, induces antihypernociceptive effects. Interrupting delivery of minocycline results in immediate return of allodynia and hyperalgesia (Hains and Waxman 2006). Minocycline treatment, applied shortly after T13 spinal cord hemisection, another model of spinal cord injury, suppresses the development of mechanical allodynia and thermal hyperalgesia (Marchand et al. 2009).

Supraspinal administration of minocycline, directly into the thalamic ventral posterolateral nucleus, induces antihyperalgesic effect induced by chronic constriction of sciatic nerve (Leblanc et al. 2011) or intrathalamic injection of recombinant chemokine (C–C motif) ligand 21 (CCL21) in rats (Zhao et al. 2007b).

Recently, it was shown that minocycline inhibits hypernociception in a cancer-induced bone pain model (Wang et al. 2011). This study that intrathecal minocycline inhibits mechanical allodynia and ongoing experimental pain in rats after carcinoma cell inoculation. Minocycline inhibited hypernociception at an early stage of tumor growth (from day 4 to day 6). However, at the late stage (from day 10 to day 12), intrathecal minocycline had no effect.

In sum, this compelling body of evidence indicates that tetracycline derivatives exhibit antihypernociceptive activity in a wide variety of models of inflammatory and neuropathic pain, with apparent absence of effect on physiological nociception. Moreover, it is noticeable that there is short time window for successful intervention after stimuli are applied to induce experimental pain.

Mechanisms underlying tetracycline antihypernociceptive effects

Chelating activity

Despite considerable progress in dissecting cellular and molecular mechanisms, the reason(s) why tetracyclines exhibit a wide repertoire of non-antibacterial activities is still intriguing. One could wonder that activities exhibited by tetracyclines in experimental models of pain result from interaction with specific molecular target(s) or are dependent on a non-specific effect by chelating divalent ions, particularly Ca^{2+} and Zn^{2+} . Such a mechanism is not unlikely, as Ca^{2+} controls many cellular functions by acting ubiquitously as an intracellular second messenger as well as an extracellular first messenger (Brown et al. 1993; Prado 2001). In addition, Zn^{2+} is a cofactor of matrix metalloproteinases (MMPs), whose role in physiological processes and inflammatory and vascular diseases, such as atherosclerosis, angiogenesis, oxidative stress and ischemia/reperfusion injury, has been under intense investigation (Chow et al. 2007; Hu et al. 2007a). Tetracyclines inhibit MMP activity, and this effect is associated with chelation of Zn^{2+} (Golub et al. 1998). However, the demonstration that PMIN induces antihypernociceptive (Bastos et al. 2008) effect suggests that this is unrelated to Ca^{2+} and Zn^{2+} chelating properties of tetracyclines.

Reinforcing this evidence, some studies have shown that some effects induced by tetracycline derivatives are little to no dependent on interaction with Ca^{2+} . It has been shown that the suppression of neutrophil functions by tetracycline is only partially dependent on Ca^{2+} concentration (Gabler and Creamer 1991). Pruzanski et al. (1992) showed that the inhibition of phospholipase A_2 (PLA_2) activity by minocycline is not reversed by excessive addition of Ca^{2+} .

Moreover, the preventive effect induced by minocycline on glutamate neuronal apoptosis is not dependent on blockade of its ionotropic receptors or reduction of subsequent Ca^{2+} intracellular rises (Pi et al. 2004).

Anti-inflammatory activity: targeting glial and other immune cells

As chelating activities seem not to help explain pleiotropic activities, one could wonder then that tetracyclines inhibit a first step in an inflammatory process or an upstream inflammatory enzyme or transcription factors which are expressed ubiquitously, thus preventing the trigger of a long cascade of cellular and molecular events. However, there is no strong evidence supporting this hypothesis. Instead, different studies have shown that antihypernociception is associated with different putative targets (Fig. 2), but the mechanism(s) proposed in each study may be simply a single step of a multi-step process or just an epiphenomenon.

Many studies have associated antihypernociceptive effects with inhibition of microglial cell activation and regarded minocycline as a “selective” or even “specific” microglial inhibitor. For this reason, minocycline has been widely used as a pharmacological tool to inhibit microglial cell activation, a key event involved in the central neural sensitisation induced by many noxious stimuli (Watkins and Maier 2003; Milligan and Watkins 2009). However, it has been shown that minocycline and other tetracyclines act on other cell types, such as T lymphocytes, macrophages and neurons (Amin et al. 1996; Brundula et al. 2002; Alano et al. 2006; Kim et al. 2011). Therefore, it is important to note that inhibition of peripheral macrophage activation is very likely to be an important mechanism underlying antihypernociception induced by systemic minocycline in the models in which these cells play an important role in peripheral neural sensitisation.

Since immune and neuronal cells produce a plethora of mediators such as prostanoids, cytokines, chemokines and growth factors (Milligan and Watkins 2009; Sorkin and Schäfers 2007), which can sensitise or directly activate neurons in the central or peripheral nervous systems, inhibition of these mediators may underlie tetracycline antihypernociceptive effects. Moreover, possible direct actions of tetracyclines on neuronal electrophysiological properties cannot be ruled out and thus this will be discussed (Kim et al. 2011).

Prostaglandin (PG)- E_2

Conventional tetracyclines or CMTs affect the activities or gene expression of enzymes in the arachidonic acid pathway. Intrathecal injection of LPS (Saito et al. 2010), and sciatic nerve (Ma et al. 2010) or spinal cord (Zhao et al. 2007a) injuries lead to microglial cell activation and

enzyme downstream to COX-2) expression in primary rat microglial cells.

Inflammatory cytokines and chemokines

Conventional tetracyclines or CMTs reduce the production of inflammatory cytokines in a wide range of animal models. Antihypernociceptive effects induced by tetracyclines are associated with reduced inflammatory cytokines production in models of inflammatory and neuropathic pain.

In the spinal immune activation model, in which allodynia is induced by intrathecal HIV-1 gp120 protein, the anti-allodynic effect induced by minocycline is associated with decreased microglial activation, reduced mRNA for IL-1 β , tumour-necrosis factor (TNF)- α , IL-1 β converting enzyme and IL-1 receptor antagonist in lumbar dorsal spinal cord, and reduced IL-1 β and TNF- α concentrations in the cerebrospinal fluid (Ledeboer et al. 2005). IL-6 concentrations in the spinal cord or cerebrospinal fluid were unaffected in this experimental setting. If the central sensitisation is induced by intrathecal LPS or injection of CFA into the gastrocnemius–soleus muscle, minocycline antiallodynic effect is also associated with reduced TNF- α concentration in the cerebrospinal fluid (Saito et al. 2010) or in the L4–L5 dorsal horn segments (Chacur et al. 2009).

Raghavendra et al. (2003) showed that the intrathecal preemptive treatment with minocycline induces antiallodynic and antihyperalgesic effects in rats submitted to spinal nerve transection, and these effects are associated with reduction of gene expression and concentrations of the inflammatory cytokines IL-1 β , IL-6 and TNF- α in the L5 lumbar spinal cord segment. Post-injury minocycline treatment inhibits only IL-1 β and TNF- α , but to a lesser extent than preemptive treatment. Spinal concentrations of IL-1 β or TNF- α are also reduced by minocycline in a model of diabetic neuropathic pain (Talbot et al. 2010). The serum concentration of IL-6 is increased 14 days after chronic constriction injury, and this effect is inhibited by systemically applied minocycline starting 1 h before surgery and continued daily until day 14 post-surgery (Zanjani et al. 2006).

Fractalkine (CX₃CL1) is a chemokine which is tethered to the extracellular surface of primary afferent neurons or intrinsic neurons in the spinal cord, whereas its receptor (CX₃CR1) is predominantly expressed in microglia. CX₃CL1, which is the only chemokine reported to be constitutively expressed in neurons, has been proposed to be a selective signal from neurons to microglia in spinal cord (Milligan et al. 2004; Verge et al. 2004). Intrathecally injected CX₃CL1 induces hypernociception, and this chemokine is endogenously released in the spinal cord after peripheral neuropathy. Minocycline prevents the mechanical allodynia and thermal hyperalgesia induced by intrathecal CX₃CL1 in rats (Milligan et al. 2005).

Supraspinal microglial cells are also involved in nociceptive processing. The injection of CCL21 directly into the thalamic ventral posterolateral nucleus activates microglia and induces mechanical allodynia and thermal hyperalgesia in rats. The nociceptive response induced by this chemokine is also inhibited by minocycline (Zhao et al. 2007b).

Brain-derived neurotrophic factor (BDNF)

There is increasing evidence that microglial cells play an important role in neuronal disinhibition in spinal nociceptive processing, a key event involved in the persistence of pathological pain (Beggs and Salter 2010). Peripheral nerve injury leads to increased release of ATP from microglia (Tsuda et al. 2003), which in turn leads to microglial BDNF release via P2X₄ receptor activation, a phenomenon which is dependent on extracellular Ca²⁺ and p38 mitogen-activated protein kinase (MAPK) activation (Trang et al. 2009). BDNF shifts the polarity of neuronal gamma-amino butyric acid (GABA) currents from inhibitory to excitatory (Coull et al. 2005) via modulation of K⁺-Cl⁻ co-transporter 2 (KCC2) (Wardle and Poo 2003; Rivera et al. 2004).

Spinal application of BDNF induces microglia activation and long-term potentiation (LTP) of C-fibre-evoked field potentials (Zhou et al. 2008, 2011). Intrathecal minocycline reduces microglia activation and blocks BDNF-induced LTP, whereas baseline synaptic transmission is unaffected (Zhou et al. 2011). On the other hand, Morgado et al. (2011) showed that in a diabetes 1-related neuropathic pain model, antiallodynic effects induced by minocycline are associated with decreased KCC2 expression in spinal cord, but without alteration of BDNF concentration.

Neuropeptides

Hemokinin-1 is a member of the tachykinin family that binds to NK1 receptor with high affinity and is involved in nociception. Chronic constriction injury induces hemokinin-1-encoding gene expression in the dorsal spinal cord. Intraperitoneal administration of minocycline inhibited the thermal hyperalgesia and mechanical allodynia and reduced the expression of hemokinin-1-encoding gene in the dorsal spinal cord (Matsumura et al. 2008). In addition, in a model of water avoidance stress, which is associated with sustained visceral hyperalgesia, minocycline also reduces the increased expression of spinal NK1 receptors (Bradesi et al. 2009). Moreover, in thoracic spinal cord of streptozotocin-diabetic rats, minocycline inhibits the increased expression of kinin B₁ receptor, which could be associated with mechanical and cold allodynia in this experimental model (Talbot et al. 2010). These pharmacological effects might contribute to the beneficial effect of minocycline in neuropathic pain.

Macrophages, neutrophils and T cells invade dorsal root ganglia after nerve injury, and this is important for the development of experimental neuropathic pain (Hu and McLachlan 2002; Hu et al. 2007b; Morin et al. 2007). Mika et al. (2010) showed that preemptive and repeated intraperitoneal injection of minocycline inhibits the trafficking of peripheral immune cells into the dorsal root ganglia after chronic constriction of sciatic nerve. This effect is associated with reduction of the pro-nociceptive peptides prodynorphin and pronociceptin in the dorsal root ganglia.

Intracellular signalling pathways affected by tetracyclines

Different studies have investigated the intracellular pathways that might mediate the multiple actions of tetracyclines in inflammation and pain. In fact, some molecular targets have been suggested. Recently, Szeto et al. (2011) have shown that in human CD4⁺ T cells, minocycline reduces nuclear factor of activated T-cells 1 (NFAT1) activation, which is a key regulatory factor in T cell activation. This reduction in NFAT1 activation might be due to the increased glycogen synthase kinase-3 activity and attenuation of intracellular Ca²⁺ influx induced by minocycline.

It has been shown that tetracyclines could alter nuclear factor (NF)- κ B activation, which is an important step in the expression of different inflammatory mediators. Minocycline and doxycycline reduce NF- κ B activity in THP-1 cells stimulated with sonicated *Borrelia burgdorferi* (Bernardino et al. 2009). Moreover, minocycline also inhibits NF- κ B activation in microglial cells (Si et al. 2004; Nikodemova et al. 2006), though this effect has not been observed in human CD4⁺ T cells (Szeto et al. 2011).

Microglia express low levels of major histocompatibility complex class II (MHCII) in non-activated form, but this expression increases when these cells are stimulated. In interferon γ -stimulated microglial cells, the increased expression of MHCII is reduced by minocycline. This reduction in MHCII expression might be due to a reduction of PKC α/β activation and PKC α/β and interferon regulatory factor 1 translocation to the nucleus (Nikodemova et al. 2007). Moreover, the anti-inflammatory effect of CMT-3 might involve the inhibition of PKC α and PKC δ activity (Sandler et al. 2005).

Minocycline potentiates nerve growth factor (NGF)-induced neurite outgrowth in PC12 cells (Hashimoto and Ishima 2010). Minocycline increases the levels of the eukaryotic translation initiation factor eIF4AI protein, a factor that is required for the binding of mRNA to the 43S ribosomal complex and for the translation process. The incubation of these cells with eIF4AI RNAi reduces the potentiating effects of minocycline on NGF-induced neurite outgrowth. Moreover, the effect induced by minocycline on NGF-induced neurite outgrowth is also reduced by an IP3 receptor blocker

(Hashimoto and Ishima 2010). This indicates that these two pathways might also be important targets for the pleiotropic effects induced by minocycline.

In addition to these effects, minocycline might also affect MAPKs. Nikodemova et al. (2006) have shown that minocycline inhibits the activation of p38 MAPK, extracellular signal-regulated protein kinases, c-Jun-N-terminal kinase (JNK) in BV-2 microglial cells stimulated with LPS, P2X₇ receptor agonist or PKC activator.

Although some intracellular signalling mechanisms have been proposed to explain the main actions of minocycline in modulating nociceptive transmission, the most studied target is p38 MAPK. This kinase is activated in microglia and neurons after peripheral nociceptive stimuli and might contribute to inflammatory and neuropathic pain (Jin et al. 2003; Svensson et al. 2003; Roberts et al. 2009). Inhibitors of p38 MAPK induce antihypernociceptive effects in different experimental models. Importantly, different studies demonstrated that minocycline inhibits p38 MAPK and microglia activation in different animal models of pain (Hua et al. 2005; Piao et al. 2006; Bradesi et al. 2009; Chang and Waxman 2010).

More non-glia effects: possible direct effects on neurons

Minocycline, at nanomolar concentration range, induces neuroprotection through direct anti-inflammatory effects on neurons. It was shown that minocycline inhibits poly (ADP-ribose) polymerase-1 (PARP-1) activation in cultured cortical neurons induced by DNA damage or oxidative stress (Alano et al. 2006). This enzyme promotes DNA repair under stress conditions and induces effects on the gene transcription by interactions with transcription factors, notably NF- κ B, which can lead to expression of several inflammatory mediators and enzymes involved in their synthesis, including cytokines, COX-2 and inducible nitric oxide synthase (Cuzzocrea et al. 2002). Consistently with these observations, it was demonstrated that other PARP-1 inhibitors, including nicotinamide and 3-aminobenzamide, also exhibit anti-inflammatory activity (Cuzzocrea et al. 1999). In cultured neurons subjected to oxygen and glucose deprivation, minocycline reduces cell death with concomitant reduction of nitric oxide concentration (Huang et al. 2010).

The excitability of neurons may also be directly affected by minocycline (Cho et al. 2006; Gonzalez et al. 2007; Kim et al. 2011). Minocycline depresses glutamatergic neurotransmission in hippocampal neurons (Gonzalez et al. 2007). It is possible that this effect may be relevant to minocycline antihypernociceptive effect as intrahippocampal injection of NMDA receptor antagonists inhibits the licking behaviour induced by formalin in rats (McKenna and Melzack 2001; Soleimannejad et al. 2007).

Cho et al. (2006) showed that antihypernociceptive effect induced by minocycline in the rat formalin test is associated with inhibition of substantia gelatinosa neuronal synaptic currents in the spinal dorsal horn, whereas the electrical properties of dorsal root ganglia neurons are unaffected. Using a different methodological approach, a more recent study showed that minocycline, at nanomolar range, does reduce the amplitudes of both tetrodotoxin-sensitive and -resistant Na^+ currents in dorsal horn ganglia neurons (Kim et al. 2011). Tetracycline also induces such an effect, but only at higher concentration range. However, minocycline slows inactivation and speeds up the recovery from inactivation. The later effects should rather enhance excitability and support the high frequency firing of the sensory neurons. At micromolar concentration of minocycline, however, Na^+ currents are entirely blocked. Therefore, the latter effects may have no consequence at a higher concentration. Taken together, these results suggest that minocycline may directly affect excitability of primary afferent neurons and this also helps explain antihypernociceptive effects. However, these results do not rule out a direct effect on glial and satellite background cells.

Interaction between minocycline and morphine

Recent studies have shown that minocycline attenuates tolerance to antinociceptive effects induced by repeated administration of morphine and enhances morphine-induced antinociceptive effect in different animal pain models (Cui et al. 2008; Hutchinson et al. 2008a, 2010; Habibi-Asl et al. 2009; Mika et al. 2009; Lewis et al. 2010). For this reason, it has been hypothesised that a combination of morphine and minocycline may have therapeutic usefulness in order to reduce morphine dose, with consequent reduction of incidence of untoward effects (Chen et al. 2010).

Repeated intrathecal injections of morphine in rats lead to tolerance, a phenomenon that is attenuated by concomitant treatment with intrathecal minocycline, at a dose which is not sufficient to induce antihypernociceptive effect for itself. The same effect is observed if minocycline treatment is started few days after starting morphine treatment, but not if the tolerance is completely established (Cui et al. 2008). Minocycline attenuates tolerance to antinociceptive effect induced by morphine also when mice received both drugs systemically or via intracerebroventricular route. The development of tolerance in mice submitted to chronic constriction injury is delayed from day 6 to day 11 after treatment if morphine is given concomitant with intraperitoneal minocycline or pentoxifylline (Mika et al. 2009). The effects induced by these two drugs were associated with inhibition of microglial activation. Central administration of minocycline suppresses the development of morphine tolerance assessed in the hotplate model (Habibi-Asl et al. 2009).

Although the mechanisms associated with morphine tolerance are not entirely clear, recent studies have provided considerable progress in revealing mechanisms underlying this phenomenon. Among these mechanisms, p38 MAPK activation in spinal microglial cells is regarded as an important one (Chen and Sommer 2009). Cui et al. (2008) showed that the effect induced by minocycline on morphine tolerance is associated with attenuation of spinal microglial activation and p38 MAPK phosphorylation induced by this opioid. In addition, it is possible that other kinase proteins are involved in this phenomenon. For instance, JNK, another enzyme from the MAPK family, and PKC activation plays a role in morphine tolerance (Mao et al. 1994; Chen and Sommer 2009). In fact, PKC is upstream to MAPK activation in the signal transduction pathway activated by morphine binding to μ opioid receptors (Chen and Sommer 2009). Consistent with this hypothesis, it was already shown that tetracyclines inhibit JNK and PKC activities (Webster et al. 1994; Nikodemova et al. 2006, 2007). Therefore, effects on other kinases may underlie minocycline actions on morphine tolerance.

NMDA receptor activation is also involved in the plastic changes which occur after chronic morphine administration and contribute to tolerance development (Trujillo and Akil 1991). Thus, the inhibitory effect induced by minocycline on glutamatergic neurotransmission (Gonzalez et al. 2007) may represent an additional mechanism contributing to its effect on morphine tolerance. Another mechanism proposed to explain tolerance to morphine antinociceptive effect is induction of apoptosis in the central nervous system (Mao et al. 2002). Indeed, it has been recently shown that minocycline inhibits this morphine-induced effect as well (Hassanzadeh et al. 2011).

It was shown that minocycline enhances the antihyperalgesic effect induced by morphine and reduces the morphine-induced upregulation of COX-1 gene expression in rat microglial cells *in vitro* in a concentration-dependent manner (Hutchinson et al. 2008a). Since systemic administration of naloxone (10 mg/kg) does not antagonise the antinociceptive effects induced by minocycline in the formalin test, it is unlikely that minocycline induces such an effect by binding to opioid receptors (L.F.S. Bastos, unpublished data). It remains to be elucidated whether the interaction between minocycline and morphine to enhance morphine-induced antinociceptive effect is potentiation, additivity or synergism. A detailed isobolographic analysis would be required for a clear elucidation.

As minocycline attenuates the tolerance to antinociceptive effect induced by morphine, it has also been investigated whether other untoward effects induced by morphine, such as respiratory depression and reward, and opioid withdrawal ethological signs are affected by minocycline (Hutchinson et al. 2008a). This study showed

that minocycline suppresses the respiratory depression and conditioned place preference induced by morphine. Hutchinson et al. (2009) also demonstrated that minocycline or ibudilast, another inhibitor of microglial activation, reduces the behavioural signs of naloxone-precipitated morphine withdrawal in mice.

Minocycline as a prototype to develop both novel antibacterial and anti-inflammatory compounds

Minimum structural requirements for antibacterial activity seem absolutely distinct from those for antihypernociceptive activity. Minocycline derivatives with chemical modifications at lower periphery (C1 and C12; Fig. 1) of the molecule, in spite of having their antibacterial activity markedly reduced, still exhibits anti-inflammatory activity. Indeed, PMIN does not inhibit the growth of minocycline-sensitive bacterial strains (Lertvorachon et al. 2005; Bastos et al. 2008), but does inhibit licking behaviour in mice to the same extent as those induced by minocycline (Bastos et al. 2008). On the other hand, tigecycline, which differs from minocycline by the long side chain at the 9 position of carbon atom (9-*tert*-butyl-glycylamido moiety) and broader antibacterial spectrum, inhibits the production of inflammatory cytokines in different experimental settings, and this effects are unrelated to the antibacterial activity (Saliba et al. 2009; Salvatore et al. 2009). Both PMIN and tigecycline are equipotent to minocycline in reducing the microglial production of PGE₂ induced by LPS (L.F.S. Bastos, unpublished data). Altogether, the different structural requirements for antibacterial and antihypernociceptive activities of tetracyclines derivatives indicate that minocycline may be a good prototype to develop new antibacterial and anti-inflammatory compounds.

Pharmacokinetic and toxicological profiles of minocycline relevant to preclinical investigation

It is noteworthy that some pharmacokinetic studies provided important information about the timing for administering minocycline relative to performing tests in animal models of acute pain. In most of the studies which have evaluated antihypernociceptive or neuroprotective effects induced by minocycline, the drug was injected at high doses via intraperitoneal route. Fagan et al. (2004) showed that the absorption of minocycline after intraperitoneal administration is prolonged in rats. Time to reach peak concentration is 2.5 h, and there is no clear elimination over a 6-h period of sampling. Furthermore, minocycline brain concentrations increase gradually until 4 h after per os or intravenous administration in rats (Colovic and Caccia 2003). Therefore,

information about minocycline pharmacokinetic profile should be cautiously considered before determining when minocycline is administered.

Doses used should also be cautiously taken into account. Minocycline is a known sclerosing agent (Light et al. 1994) and may cause tissue damage and scarring when administered repeatedly directly into the peritoneum. However, treatment of rats with a low dose seems to be safe because important physiological parameters are not altered. Intraperitoneally administered minocycline (45 mg/kg twice a day in the first day; 22.5 mg/kg for the subsequent 2 days) induces neuroprotective effect without affecting rectal temperature, arterial blood pressure, plasma glucose, or arterial blood gases (Yrjanheikki et al. 1999). Doses higher than 25 mg/kg induce body mass reduction, skin irritation, rise of hepatic enzymes and triglycerides and a slight decrease in potassium excretion (Noble et al. 1967; Bocker et al. 1991; Smith et al. 2003). Minocycline (100 mg kg⁻¹ day⁻¹ for 35 days) induces thyroid pigmentation associated with functional deficits at a high dose (Tajima et al. 1985). In experimental animals, minocycline is lethal (DL₅₀, 3,600 mg/kg) at doses that are irrelevant to studies on pain and neurodegeneration (Blum et al. 2004).

Potential higher safety of CMTs

The interest in the CMTs derives from potential superior safety. These derivatives would not induce the development of tetracycline-resistant microorganisms after prolonged use. A clinical trial to evaluate the incidence of untoward effects induced by a high dose of minocycline in the treatment of acne (mean duration of 10.5 months) showed that this treatment causes candidiasis and gastrointestinal disturbance (Goulden et al. 1996). These untoward effects may reduce patient compliance to prolonged treatments.

It is also possible to narrow tetracycline pleiotropic actions by modifying molecule sites related to chelation of Ca²⁺ and Zn²⁺. As previously mentioned, tetracyclines are absolutely contraindicated to children younger than 9 years and to pregnant and nursing women. This absolute contraindication is due to their high affinity to developing teeth and bones, with consequent accumulation in these tissues, possibly causing unsightly cosmetic and functional defects, such as teeth discoloration, gum dysplasia, dental hypoplasia or bone deformities. Affinity to these tissues is likely to be caused by formation of a tetracycline–Ca²⁺ orthophosphate complex. The prevalence of tetracycline and minocycline teeth and oral cavity staining is 3–6% (Sanchez et al. 2004). The mechanism of minocycline staining is still unknown, but one can elucidate whether this effect is due to formation of tetracycline–Ca²⁺ orthophosphate complex by

investigating the effects induced by non-chelating derivatives, being potentially useful pharmacological tools. Moreover, non-chelating derivatives may be desirable when inhibition of physiological activities exhibited by MMPs is untoward. Therefore, toxicological studies are needed to investigate the potential superior safety of CMTs over conventional tetracyclines.

Perspectives

Minocycline is a safe generic medicine which has been clinically used since the 1970s, and whose pharmacokinetic and toxicological profiles are well known. However, antibacterial effect is a limitation of its prolonged use by patients with chronic painful diseases. Only one study showed antihypernociceptive effect induced by a CMT devoid of antibacterial activity, which is derived from minocycline (Bastos et al. 2008), though its effects in neuropathic pain models remain to be tested. Regarding their potential favourable safety profile, and ease to produce with reasonably low cost, CMTs are promising compounds to be screened as putative tools for neuropathic pain management. In combination with opioids, they might be useful for enhancing their antinociceptive activity and reducing the incidence of untoward effects, as minocycline has been shown to induce such beneficial effects (Hutchinson et al. 2008a, 2009, 2010; Chen et al. 2010; Lewis et al. 2010). Such positive interaction is expected as pilot clinical studies have shown that oral low dose of naltrexone, another drug that has also been shown to inhibit activation of glial cells (Hutchinson et al. 2008b; Mattioli et al. 2010), reduces fibromyalgia symptoms in patients not receiving opioids (Younger and Mackey 2009) and enhances analgesic effect induced by continuous intrathecal morphine for chronic pain (Hamann and Sloan 2007).

There is a paucity of information on the relationship between chemical structure and antihypernociceptive activity played by tetracyclines. Creation of chemical libraries of tetracyclines for screening therapeutic agent candidates is promising. A better understanding of the mechanisms underlying tetracyclines pleiotropic activities, as well as the structure–activity relationship of both antibacterial and non-antibacterial tetracycline derivatives, may open new opportunities for the development of pharmacological tools useful for basic research or for managing painful pathological conditions, especially those whose management is more challenging. This would be one of the clever ways to take advantage of the versatility of this chemical class of compounds.

Acknowledgements We acknowledge Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Ministry of Education, Brazil), Conselho Nacional de Desenvolvimento Científico e

Tecnológico (CNPq, Ministry of Science and Technology, Brazil), Fundação de Apoio à Pesquisa do Estado de Minas Gerais (FAPEMIG, Minas Gerais, Brazil) and Pró-Reitoria de Pesquisa (PRPq) da UFMG for constant support.

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