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Review Article

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Non-Antibacterial Implications of Tetracyclines in the Management of Periodontitis: An Update

Bangalore Varadhan Karthikeyan[1]*, Devireddy Venkata Tejaswi[1] and Munivenkatappa Lakshmaiah Venkatesh Prabhuji[1]

Abstract

Recent research has shown that the majority of the soft and hard tissue destruction in periodontitis occurs due to activated host immune-inflammatory defence mechanisms in response to bacterial plaque. The importance of the host inflammatory response in periodontal pathogenesis presents opportunity for exploring new treatment strategies for periodontitis by means of host modulation. One of such treatment strategy is the use of drugs like Chemically Modified Tetracyclines, which are derivatives of tetracycline group of antibiotics, but lack antimicrobial action and have potent host modulating properties. The main advantage of Chemically Modified Tetracyclines over conventional tetracyclines is that they do not produce the side effects such as gastro-intestinal toxicity and antibiotic resistance. Hence, host modulation with Chemically Modified Tetracyclines can be considered as a viable adjunctive treatment modality in the management of periodontitis. The present review focuses on the current status and limitations of Chemically Modified Tetracyclines as a host modulating agent in the management of periodontitis.

Keywords

Periodontitis; Host modulation; Chemically modified tetracyclines; Antibiotic resistance

Introduction

Periodontitis has been considered to be caused by specific bacteria or groups of periodontopathic bacteria (specific plaque hypothesis) [1]. It is also speculated that it is due to non-specific accumulation of dental plaque, which might lead to environmental changes in the gingival sulcus causing an immune mediated tissue changes and further fostering environmental changes that contribute to a greater degree of tissue damage (ecological plaque hypothesis) [2,3]. Based on this hypothesis, treatment protocols were predominately anti-infective therapies. However, during 1980's research began to focus very closely on host-bacterial interaction, leading to "host-bacterial interrelationship" era [4]. During this era, it was recognized that the development and progression of periodontal disease is largely based on the host immune-inflammatory responses, and not on specific bacteria or their putative virulence factors. This implication of the paradigm shift on the concentration of host response has led to the development of Host Modulation Therapy (HMT). HMT is a treatment concept that aims to reduce tissue destruction by down regulating the destructive aspects of the host response and up regulating the protective or regenerative component [5]. The concept of HMT was first introduced in dentistry by Williams RC and Golub LM in 1990.

With this current understanding of the host response in periodontal disease pathogenesis, it is intuitive that pharmaceutical inhibition of host response pathways may be an adjunctive or alternative strategy for treating periodontal diseases [6]. As stated in the position paper of American Academy of Periodontology (AAP) 1992 "blocking host responses involved in the periodontal disease progression in addition to controlling the etiological bacteria, is an emerging concept in periodontics." In this regard, therapeutically modulating host response agents have surfaced. One such host modulation agent is chemically modified tetracyclines. Chemically Modified Tetracyclines (CMTs) are derivatives of tetracycline group of drugs, which lack antimicrobial action but potentially down-regulates the activity of MMPs [7,8]. CMTs are being investigated as potential host modulating agents in the management of periodontitis, but currently they are not approved by FDA. The objective of this review is to bring the reader up to date on the current understanding of the host-modulating agent like CMTs and their potential benefits and limitations in the management of periodontitis.

The Matrix Metalloproteinases (MMPs)

Among the host-derived proteases, MMPs play a significant role in the destruction of connective tissue in periodontal diseases. MMPs (secreted and membrane-bound) are an important family of zinc- and calcium-dependent endopeptidases secreted or released by a variety of host cells that function at neutral pH and utilize the various constituents of the extracellular matrix including collagen, gelatin, proteoglycans, fibronectin, laminin and elastin as their substrates [9]. These proteinases are involved in a number of physiological and pathological events. Physiological events include cell migration, wound healing, tissue remodelling and tooth eruption, where as pathological processes include periodontal disease, arthritis, cancer, atherosclerosis, diabetes, pulmonary emphysema and osteoporosis [10,11].

Majority of the cell types found in human periodontal tissues including fibroblasts, keratinocytes, macrophages, neutrophils, and endothelial cells produce MMPs. In healthy tissues, MMPs are produced primarily by fibroblasts (MMP-1 or collagenase-1) and are concerned with the maintenance of the periodontal connective tissues [12]. PMN type MMPs (MMP-8, MMP-9) and bone-derived MMP (MMP-13) are known to be important in periodontal disease progression [12], and their transcription is up-regulated by pro-inflammatory mediators such as interleukin- 1α and β (IL-1α and β) and tumour necrosis factor-α(TNF-α) [13]. The levels of PMN-type MMPs correlates with the disease severity i.e., increase with periodontal disease severity and decrease following therapy [14]. In physiologically healthy tissues, MMPs are self-regulated by their own proteolytic inactivation and by endogenous inhibitors like α2-macroglobulin (α2M) and tissue inhibitors of MMPs (TIMPs), the major plasma inhibitor of MMPs [10]. The role of inhibitors is particularly important because an imbalance between the activated

***Corresponding author:** Bangalore Varadhan Karthikeyan, Department of Periodontics, Krishnadevaraya College of Dental Sciences, Hunasamaranahalli, via Yelahanka, Bangalore- 562157, India, E-mail: drkarthikeyanbv@rediffmail.com

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matrix metalloproteinases and their endogenous inhibitors leads to pathological breakdown of the extracellular matrix [6].

This shows that a systemic pharmaceutical agent that could down-regulate the pathologically elevated levels of MMP activity could be extremely useful as an adjunctive treatment in periodontitis. Such a rationale has led to the development of synthetic matrix metalloproteinase inhibitors as potential therapeutic agents in treatment of periodontitis. Chemically modified tetracyclines (CMTs) are one such group of drugs, which have shown to inhibit pathologically elevated MMPs, and pro-inflammatory cytokines [7,8].

Development and Structure of Chemically Modified Tetracyclines

CMTs possess the ability (an ability shared by all members of tetracycline family) to down-regulate MMP activity. This ability of CMTs to inhibit the breakdown of connective tissue by a non-antimicrobial mechanism was first reported in 1983-84 [15-17]. Golub and co-workers during experiments in diabetic rat model observed the rapid increase in collagenase activity in gingiva of severely hyperglycemic diabetic rats and subsequent severe periodontal destruction attributed to increase in collagenolytic activity (indicates an altered host response). Tetracycline therapy was brought into the experimental diabetes protocol, to eliminate the oral microbial factors. Beside the antibacterial property, tetracyclines were able to inhibit the collagenase activity. As a result of these initial studies, Golub et al. proposed that tetracyclines could inhibit collagenase by a mechanism independent on the drug's antibacterial efficacy [15]. Further, they recognized that the anti-microbial and anti-collagenase properties of tetracyclines resided in different parts of the four-ringed structure and alterations in the structure of parent tetracyclines led to the development of new family of tetracycline compounds known as chemically modified tetracyclines (CMTs) [18].

All tetracycline compounds show a basic chemical structure consisting of a tetracyclic naphthacene carboxamide ring system. The antimicrobial activity of tetracyclines is resided in the dimethylamine group at carbon-4 (C4) position side-chain in ring A. The alterations in the tetracycline molecule involved the removal of the dimethylamino group from the carbon-4 position of the "A" ring (but to retain its zinc-binding β-diketone site) resulting in the CMT called 4-de-dimethylaminotetracycline (CMT-1). This resulting compound, 4-de-dimethyl amino tetracycline (CMT-1) did not have antimicrobial property but have potent anti-collagenase activity both in vitro and in vivo [7].

Since, the first CMT was described in 1987; more than 30 different CMTs are synthesized by addition or deletion of functional groups to the parent tetracycline molecule [18]. Chemical structures of some of them are CMT-1 (4-dedimethylaminotetracycline), CMT -2 (tetracyclinonitrile), CMT-3 (6-deoxy-6-demethyl-4-de-dimethylaminotetracycline), CMT-4 (7-chloro-4-de-dimethylaminotetracycline), CMT-5 (tetracycline pyrazole), CMT-6 (4-dedimethyl amino. 4-hydroxytetracycline), CMT-7 (12 -deoxy-4-dedimethyl amino tetracycline) and CMT-8 (4-dedimethylamino doxycycline). The anti-collagenase action of the CMTs is due to the Ca^{2+} and Zn^{2+} binding sites at the carbonyl oxygen and the hydroxyl groups of carbon-11 and carbon-12 positions. CMT-5 lacks anti-collagenase property due to the replacement of carbonyl oxygen at C-11 and the hydroxyl group at C-12 by nitrogen atoms. Therefore, CMT-5 is inactive against MMPs [19,20]. This suggests that presence of these side chains on the tetracycline molecule is crucial for anti-collagenase property of CMTs [19,20].

Potential Benefits of Chemically Modified Tetracyclines in Periodontal Therapy

Traditionally, tetracyclines have been advocated as useful adjuncts in periodontal therapy. This was based on its perceived advantages like high anaerobic activity, high gingival crevicular fluid (GCF) concentration compared to serum, and their ability to bind to the tooth surface, followed by slow release [20]. Conversely, in spite of its potential benefits, its usage on a broad basis declined due to the widespread bacterial resistance attributed to its antimicrobial property. This limitation was overcome with the advent of CMTs, which are now recognized to have the non-antimicrobial properties that have therapeutic implication in host modulation [20,21]. The key issues related to biological roles and adverse effects of CMTs are listed in Table 1.

Anti-collagenolytic properties

The ability of tetracyclines to inhibit MMPs has provided the therapeutic applications for treating a variety of tissue destruction diseases including periodontitis. CMTs inhibit the MMPs by binding to the Zn^{2+} ions at the active site and to a lesser extent to Ca^{2+} ions in the MMP molecule. This property is supported by the following explanations; firstly, the inhibition can be reversed by adding excess of zinc or calcium [15] and secondly, tetracycline derivates, which lacks structural elements for metal binding cannot inhibit MMPs [19]. [c]MMPs contain secondary Zn^{2+} and Ca^{2+} ions outside the catalytic domain of the enzyme, in addition to those within this domain. These secondary ions help to maintain the conformity and catalytic activity of MMPs. Tetracyclines have shown to interact with these secondary metal ions in particular Zn^{2+}. In this way, tetracyclines alter the conformity of the MMPs and block its catalytic activity. In vitro experiments have shown that tetracyclines are non-competitive inhibitors of MMPs [22].

The anti-collagenase activity of CMTs is specific against the

Table 1: Biological roles of CMTs and their adverse effects.

Biological roles of CMTs	• Inhibits MMP-2, MMP-8, MMP-9, MMP-13. • Inhibits neutrophil elastase. • Inhibits gingipains produced by *P. gingivalis*. • Prevents the proteolytic inactivation of enzymes like serpins. • Stimulates the production of osteoblast alkaline phosphatase and collagen synthesis. • Inhibits osteoclast enzymes like Tartrate resistant acid phosphstase and cathepsin –L. • Scavenges the reactive oxygen species and reactive nitrogen species. • Inhibits pro-inflammatory cytokines such as IL-6, TNF-α and PGE2. • Inhibits type IV collagenase thereby preventing the tumour cells from invading the basement membrane. • Induces the apoptosis of tumour cells.
Adverse effects of CMTs	• Photosensitizing property • Elevated liver function tests • Neurotoxicity • Systemic lupus erythematosis • Cytotoxic effects at higher concentrations

PMN MMPs (MMP-8, -9) but not on the fibroblast MMPs (MMP-1). This is because the Minimum Inhibitory Concentration (MIC) of the tetracyclines for the PMN collagenase (MMP-8) was found to be much lower than that for the fibroblast collagenase (MMP-1) [20]. This differential sensitivity of PMN and fibroblast collagenase to CMTs may have potential therapeutic benefit as they reduce pathologically elevated collagenolytic activity during inflammation, but not the collagen turnover required for maintaining normal tissue integrity [23,24]. During initial studies by Golub and co-workers, CMTs have shown to inhibit both animal and human collagenases at a concentration of 2µg/ml [7]. In experimental diabetic rats, a peak plasma concentration of 4µg/ml was attained after an optimum dose of 5 mg/day [25,26]. As opposed to parent tetracyclines, development of resistant bacteria was not reported with CMTs [22].

The potency of CMTs and doxycycline were compared and it was shown that in a rat model infected with *P.gingivalis*, CMT-1 inhibited the host collagenase to the same extent as doxycycline [27]. Furthermore, CMT-1 and doxycycline reduced the inhibitory effect on the expression of type-I and type XII collagen m-RNA expression from cells, which would enhance the collagen production in periodontitis [28].

CMTs especially, CMT-1 and -3 have shown to inhibit gingipains (cysteine proteinases) produced by *P. gingivalis,* thereby preventing the proteolytic inactivation of enzymes like serpins (serine proteinase inhibitors such as α1-macroglobulin, TIMPs and α2-macroglobulin) [29]. Furthermore, CMTs inhibit elastases particularly neutrophil elastase by 10-fold more than doxycycline [30]. However, among all CMTs, only CMT-3 has the inhibitory effect towards neutrophil elastase, not all CMTs.Thus, CMTs protect elastase susceptible substrates (such as elastic fibers, fibronectin, proteoglycans and tissue inhibitors of matrix metalloproteinases) from proteolytic attack [31]. CMT-1, CMT-3 and CMT-8 have been shown to have potential anti-collagenolytic effects.

Pro-anabolic and anti-catabolic effects on bone

CMTs have pro-anabolic effects on bone. CMTs can increase bone formation via the following mechanisms: 1) increasing both steady-state levels of type I pro-collagen mRNA and collagen synthesis [32] 2) partially restoring osteoblast activity thereby enhancing bone matrix formation and mineralization, which are depressed during disease [33] and 3) increasing the number of active osteoblasts relative to inactive osteoblasts [34].

CMTs can inhibit bone resorption induced by parathyroid hormone [35] and endotoxins [36]. CMTs as anti-resorptive drugs may act synergistic to bisphosphonates; a combination of CMT-8 along with clodronate reduces endotoxin induced alveolar bone loss in rats. This combination therapy can inhibit the conversion of latent MMPs in to their active form, this action was not seen when the drugs acted individually [36]. In addition to this, CMTs stimulate the production of osteoblast alkaline phosphatase and collagen synthesis [37]. Golub et al. in 1990 reported that tetracyclines prevent osteopenia in the streptozocin-induced diabetic rat and that this effect was associated with a restoration of defective osteoblast morphology. Thus, TCs enhanced the expression of the mature osteoblast phenotype [38].

Furthermore, CMTs can affect several parameters of osteoclast function and consequently inhibit bone resorption by multiple mechanisms, which includes (1) increasing the intracellular calcium concentration and interacting with the putative calcium receptor (ryanodine cell receptor), in addition CMT-1 has attenuated the response of nickel, a potent agonist of osteoclast calcium receptor [39] 2) CMTs inhibit collagenase activity [40]. Collagenase released from osteoclast degrades the barrier of non-calcified osteoid creating a pathway for osteoclasts to reach the underlying mineralized bone surface and initiate resorption [8] (3) decreasing ruffled border area [41,42] (4) diminishing acid production [43] (5) diminishing the secretion of lysosomal cysteine proteinases (cathepsins) [44] (6) inducing cell retraction by affecting podosomes [45] (7) inhibiting osteoclast gelatinase activity [45] (8) selectively inhibiting osteoclast ontogeny or development [45] and (9) inducing apoptosis of osteoclasts [45]. CMTs are also shown to prevent the formation of multinucleated osteoclast-like cells from its precursors [4].

CMTs have the property of substantivity by which they provide sustained drug concentrations. CMT-8 binds to the bone surface and provides a prolonged release of the drug at the site of active bone resorption, where it interacts with ryanodine cell receptors present on the nuclear membrane and alters the nucleoplasmic calcium influx, thereby affecting the osteoclast gene expression and apoptosis [46]. Further, CMTs suppress the bone resorption by inhibiting the osteoclastic enzymes like tartrate resistant acid phosphstase and cathepsin -L that degrade the organic components of bone. Cathepsin L is the most potent among the collagenolytic cathepsins and the specific activity of cathepsin L was nearly 25-fold greater than cathepsin B in cultured osteoclasts [47]. CMTs caused a significant decrease in extracellular cathepsin L activity after 3 hours [44]. Tetracyclines might inhibit bone matrix degradation by reducing the secretion rather than the activity of the collagenolytic cathepsins, particularly cathepsin L [44]. Among CMTs, CMT-1 & -8 are shown to have potent pro-anabolic and anti-catabolic effects on bone.

Scavenging the reactive oxygen species (ROS) and reactive nitrogen species (RNS)

The reactive oxygen species include oxygen-derived free radicals (e.g., superoxides, hydroxyl) and non-radical compounds (e.g., hypochlorus acid, hydrogen peroxide) [48]. CMTs may decrease the ROS burden by inhibiting PMN-derived reactive oxygen metabolites, by directly scavenging free radicals and inhibiting reactions that lead to free radical generation. CMTs inhibit the production of hypochlorus acid (HOCL), which activates the latent MMPs into active MMPs and inactivates the host-derived proteinase inhibitors α1 and α2 macroglobulins [49]. This ability may further contribute to the non-antimicrobial, anti-inflammatory properties of CMTs, thereby preventing the destructive events that occur in periodontitis.

Reactive nitrogen species include nitric oxide (NO), nitrogen dioxide radicals, and products arising from the reaction of NO with oxygen-free radicals such as peroxynitrite. Peroxynitrite is most reactive among them, and is highly cytotoxic. Further, it inhibits collagen and proteoglycan synthesis and up regulates the MMP expression thereby mediating the periodontal destruction. CMTs inhibit the expression of inducible nitric oxide synthase (iNOS) which is required for the production of peroxynitrite from NO thereby preventing the protein denaturation [50]. CMT-3 and CMT-8 have shown maximum inhibitory effect on the RNS, CMT-1 and-2 had intermediary effect while CMT-5 was ineffective.

Inhibitory effects on pro-inflammatory cytokines

The host cells produce a variety of cytokines and other inflammatory mediators in response to dental plaque biofilm. Pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, IL-8 and tumour necrosis factor (TNF)-α have shown to play an important role in periodontal tissue breakdown as they activate the latent MMPs into their active form by up-regulating their transcription [13]. CMTs inhibit release of pro-inflammatory cytokines from lipopolysaccharide (LPS) stimulated host immune cells by suppressing phosphorylation of the nuclear factor κ-B cell-signalling pathway [21].

CMT-8 selectively inhibits the expression of IL-6 (which induces osteoclasts formation and activation thereby mediating inflammatory bone destruction) in cultured osteoblastic cells [51]. Besides that, CMT-8 increases the IL-10 levels, an anti-inflammatory cytokine, resulting in reduced osteoclastogenesis and bone resorption. CMT -3 has shown to inhibit intracellular accumulation and synthesis of TNF-α in activated mast cells. It also inhibits protein kinase - C production, which mediates the transcription of MMPs. Thus, inhibition of this mediator may produce an anti-inflammatory effect [52].

CMT-3 and CMT-8 inhibited cycloxygenase-2 (COX-2) mediated PGE-2 production in vitro. The mechanism of action of the CMT-3 on COX-2 seems to be at the level of post-translational modification of COX-2 (unlike tetracyclines, which act at the level of COX-2 mRNA accumulation). CMTs have not shown any inhibitory action on COX-1 protein, but significantly inhibited prostaglandin E2 (PGE2) production by blocking COX-2 production. Thus, the mechanism of action of CMT-3 on COX-1 and COX-2 seems to be distinct [53].

Moreover, CMTs play a variety of biological roles in inflammation and wound healing. In early phases of inflammation, CMTs (at low concentrations) may modulate integrin expression on endothelial cells, thereby blocking the action of inflammatory cells such as neutrophils. Further, CMTs will counteract the effects of TGF-β1, which act as chemotactic factor for mast cells, monocytes, neutrophils and fibroblasts. They inhibit the actions of TGF-β1 induced expression of MMPs, secretion of pro-inflammatory cytokines, degradation of immunoglobulins, and expression of FcγRIII, which enhances phagocytosis. CMTs also stimulate the fibroblasts to produce matrix molecules and protease inhibitors like tissue inhibitor of metalloproteinase-1 (TIMP-1). Thus, CMTs cause shift in the local microenvironment towards an anti-inflammatory anabolic situation by favouring the matrix and collagen deposition by inhibiting or reducing MMP activity and pro-inflammatory cytokines (e.g. IL-1, IL-6, TNF and prostaglandins) [54]. Among CMTs, CMT-3 & -8 are shown to have potent inhibitory effects on pro-inflammatory cytokines.

Medical applications of CMTs

CMTs can be used in the management of variety of medical conditions, which involve excessive collagenase production. CMTs have shown to be beneficial in the treatment of rheumatoid arthritis as they suppress the excessive collagenase activity in the cultured synovial tissue. Owing to this anti-collagenase action, CMTs can act synergistic to NSAIDS in the management of rheumatoid arthritis [55]. In animal studies, CMTs (especially CMT-8) markedly reduced the severity of osteoporosis by enhancing new bone formation as well as reducing bone reabsorption [56]. In addition, CMTs are effective in controlling both type I and II diabetes mellitus by inhibiting MMP activity [57]. CMT-8 has shown to reduce non-enzymatic glycation of proteins in serum and skin, reduced proteinuria, prevented cataract formation and tooth loss in type-II diabetic rats [58].

Currently, CMTs are being investigated for their anti-metastatic actions in the tumours of prostate, breast, and melanomas [59,60]. It is shown that they inhibit type IV collagenase thereby preventing the tumour cells from invading the basement membrane [61]. In addition, the ability of CMTs to inhibit cell motility can contribute in reducing the invasive activity of malignant cells [62]. CMT-3, and its nitro derivatives have potent anti-invasive properties [63] CMTs inhibit selective osteoblast ontogeny or development and induce their apoptosis. These properties make CMT-8 potentially useful for the treatment of bone related malignancies [62].

Further, CMTs can be used in the treatment of aortic aneurysms [63], non-infected corneal ulcers [20], epidermolysis bullosa [64] and acute respiratory distress syndrome [65], which are associated with excessive collagenase activity. In addition, CMT-3 possesses anti-fungal properties [66]. CMT-3 is useful in the prevention of chemotherapy-induced painful neuropathy [67] and neuronal damage [68]. Thus, CMTs are shown to have interesting pleiotropic properties, which provide significant therapeutic potential for treating periodontitis and various other chronic inflammatory conditions.

Comparison of Chemically Modified Tetracyclines

CMTs have distinct pharmacological properties (due to different chemical structure) from each other such as lipophilicity and half-life. The most lipophilic CMTs are more efficiently absorbed from gastrointestinal (GI) tract into blood after oral dosing. The order of lipophilicities is CMT-3 > CMT-8 > CMT-1> CMT-4 > CMT-7 [27]. CMT-3 has long serum half-life that is 2.1 to 11.0 times longer than any other tetracycline compounds. This increase in half-life may reflect the lipophilicity of CMT-3 [69].

Further, a comparative evaluation of six different CMTs in inhibition of MMPs showed that the CMT-8 was most effective inhibitor of endotoxin-induced periodontal breakdown (mediated by MMP-9). CMT-8,-1,-3, doxycycline, CMT-4, -7 inhibited pro-inflammatory cytokines (IL-1, IL-6, TNF-α) and MMPs in descending order [70]. CMT-1, CMT-3, CMT-6, -7 and -8 were effective inhibitors of osteoblastic collagenase in culture. CMT-8 was the most potent among these [41]. In addition, CMT-3 and CMT-8 have shown maximum inhibitory effect on the RNS, CMT-1 and-2 had intermediary effect while CMT-5 was ineffective [50].

Among all the CMTs, CMT-3 (6-demethyl-6-deoxy-4-dedimethylaminotetracycline) is the only chemically modified, non-antimicrobial analogue of tetracycline clinically tested in humans [70,71]. Various in vivo and in vitro studies have shown that CMT-3 is more potent than most other CMTs or doxycycline (an exception is CMT-8) [41,70]. CMT-3 has been found to be a more potent inhibitor of MMP activity (lower MIC than doxycycline and other CMTs), pro-inflammatory cytokine production, and alveolar bone loss induced by *P. gingivalis* or endotoxins [70]. In addition to this, CMT-3 has shown superior pharmacokinetics including a long serum half-life after oral administration and highly lipophilic [69]. Based on these characteristics, CMT-3 has been tested in phase I and phase II clinical trials in cancer patients [72,73]. Besides CMT-3, CMT-8 has

been shown to be superior in inhibition of various MMPs such as MMP-8 and 13 and improved the bone density in ovariectomized rats [57]. CMT-8 has also demonstrated better pharmacokinetics in the rat model, when administered orally [70].

In vitro studies have shown that MIC of CMT-1 (>25 µg/ml) is much higher than doxycycline (0.25 µg/ml) [20] Therefore, MIC for doxycycline is easily attained in rat models or in humans, when administered in conventional doses, whereas the MIC for CMT-l is considerably above that achievable in rats (about 1 to 6 µg/ml) [73]. Hence, CMT-l has not yet approved for testing in humans.

Nevertheless, CMTs have shown to exert dose-dependent actions on periodontal ligament (PDL) cells. CMTs at low concentrations (10-200 µM), have shown to stimulate the MMP-2 production (up to sevenfold) by periodontal ligament (PDL) cells. In contrast, at higher concentrations (200-500 µM), gelatinase (MMP-2 and -9) activity was reduced by 10-40 folds [68]. Although CMT-5 lacks inhibitory effect on the activity of MMP-2 and MMP-9 but it showed stimulatory effect on the production of MMP-2 at the intermediate concentrations. This shows that an MMP-independent mechanism could be responsible for the stimulation of MMP production. The stimulation of MMP production by CMTs (at low concentrations) can be due to cells might sense a decrease of extracellular MMP activity and in response increases their MMP production. This study also suggests that the action of CMTs may vary in vitro and in vivo. In vitro, CMTs act directly on cells, whereas in vivo systemic properties, like the uptake of the drug from the digestive tract, also play an important role. Furthermore, at higher concentrations (500 µM), CMT- 3 and -8 reported cytotoxic effects on PDL cells. However, this effect is not seen with lower concentrations (10µg/ml). These dose-dependent actions of CMTs should be considered in their clinical use [74].

Limitations of CMTs

Even though CMTs have been reported to reduce the progression of experimentally induced periodontitis in animal models till date, only one documented short-term clinical trial on CMT-3 in patients with periodontitis has been reported in the literature, which showed reduction in MMP-8 and IL-1β in active sites [75]. One of the major limitations of CMTs usage in human studies is its photosensitizing property [71] and it is due to the photo-oxidation process, which is an oxygen-dependent pathway that involves a tetracycline photoproduct and possibly singlet oxygen [76,77]. The potential reactive sites for this singlet molecular oxygen are phenolic ring, tertiary amino groups, and conjugated double bonds in the tetracycline structure [78]. Conversely, systemic lupus erythematosis (SLE) is an unexpected side effect of CMT-3, even though it does possess neither C7 dimethylamino group, nor C4 dimethylamino group, which are responsible for this side effect [79]. Further, it also shown that CMTs have certain potential non-dose related toxicities like anemia, anorexia, constipation, dizziness, elevated liver function tests, fatigue, fever, headache, heartburn, nausea, vomiting, and neurotoxicities [71]. Besides, CMTs have shown irregular absorption rates [80], cytotoxic effects (at higher concentrations) and may cause the excessive suppression of MMPs, which can hamper the normal physiologic turnover of collagen [81].

Future Directions

Due to the fact that, the major side effects of CMTs are due to their structural limitations, it becomes imperative to carry further in vitro studies to overcome this, which are acting as a hindrance for its clinical usage in humans. Once the break through happens in obtaining the stable structure, clinical trials on humans are encouraged to utilize the potential benefits of CMTs seen in the in vitro studies for the management of periodontitis and other medical conditions. Since, CMTs have exerted clinically significant photosensitivity in a dose-dependent manner; which has led to the search for newer molecules like "PEZBINs" (poly-enolic/phenolic zinc-binding molecules) with the same active site as CMT (i.e., the β-diketone, metal ion [calcium and zinc]-binding site at carbon-11 and carbon-12), but with different phenolic super-structure [82-84]. However, these novel compounds do not belong to tetracycline family, but are shown to be effective in inhibition of MMPs, pro-inflammatory cytokines and alveolar bone loss [85]. Since, tetracycline family drugs are economical, further research should focus on developing a stable CMT molecule, so that its potentiality can be reaped on a large-scale for the management of periodontitis in developing countries.

Conclusion

Since the advent of chemically modified tetracyclines in 1987 and its proven potential, their widespread usage in humans on large scale is still far from reality, due to the structural limitations, which is attributing to its major incapacitating side effects. To date, only sub-antimicrobial dose doxycycline (an analogue of tetracycline) is the only FDA approved agent for treatment of periodontitis because of its high safety profile. Hence, future studies should aim to overcome the shortcomings of CMTs to utilize its potential benefits in humans for the management of periodontitis.

Apart from CMTs, the macrolides like azithromycin are emerging as a novel host-modulating agent in the management of periodontitis, due to their anti-inflammatory properties and their ability to concentrate and release from fibroblasts, neutrophils, and macrophages at active sites of inflammation, seemed to have a beneficial effect in the treatment of periodontal disease.

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Author Affiliation

Top

[1]*Department of Periodontics, Krishnadevaraya College of Dental Sciences, Bangalore, India*