We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chemically Modified Tetracyclines

Anshul Sawhney

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.79724>

Abstract

The use of chemotherapeutic agents or drugs specifically designed to treat periodontal diseases is emerging to aid in this risk assessment and reduction strategy. They include locally applied and systemically delivered antimicrobials and host modulatory therapies. Tetracyclines are a group of antibiotics produced naturally from certain species of *Streptomyces* or derived semisynthetically. Their advantages were broad-spectrum activity, better tolerated, and less toxic to some individuals. Further modifications of these natural products by means of synthetic reactions and reagents led to the production of the clinically used antibiotic tetracycline compounds—minocycline, doxycycline, and methacycline. Tetracyclines are now recognized to have non-antimicrobial properties that may also be therapeutically advantageous. They have anti-inflammatory properties, particularly in the treatment of certain skin diseases. One important aspect is their ability to inhibit host collagenolytic enzymes, an effect that inhibits the connective tissue degradation and thus preventing bone resorption. To identify the site of the anticollagenase property, Golub and co-workers in 1991 synthesized 10 different analogs of tetracyclines known as chemically modified tetracyclines (CMTs 1-10). All 10 analogs lacked antimicrobial efficacy and inhibited collagenase activity, but only 1 did not. Though not approved for human use by the FDA, preliminary studies using CMT-3 are being investigated on humans with cancer.

Keywords: anti-collagenase, anti-inflammatory, collagenolytic enzymes, nonantimicrobial

1. Introduction and background

Host modulation with chemotherapeutic therapy or drugs is a new adjunctive therapeutic option for the management of periodontal diseases. The concept of host modulation was introduced by Williams and Golub et al. and then expanded on by many scholars. Various

studies have indicated that host responses believed to be involved in pathogenesis of periodontal diseases may be efficacious in slowing progression of periodontitis. For the management of periodontal diseases, conventional approaches were initially mechanical in nature, as reviewed in the historical section, that is, surgery as well as scaling and root planning. New adjunctive approaches involve modulation of the host response. It has been recognized that genetic, environmental (e.g., tobacco use), and acquired risk factors (e.g., systemic disease) can increase a patient’s susceptibility to developing periodontitis. Some of these risk factors can be modified to reduce a patient’s susceptibility.

Risk assessment and therapy may include smoking cessation, improved control of diabetes, nutritional supplementation, improved oral hygiene, changes in medication, stress management, and more frequent dental visits.

The use of chemotherapeutic agents or drugs specifically designed to treat periodontal diseases is emerging to aid in this risk assessment and reduction strategy. They include locally applied and systemically delivered antimicrobials and host modulatory therapies which can be used to reduce excessive levels of enzymes, cytokines, and prostanoids as well as to modulate osteoclast and osteoblast function. Golub and colleagues discussed “host modulation with tetracyclines and their chemically modified analogs.” When considering the imbalance in destructive or pro-inflammatory mediators versus protective or anti-inflammatory mediators in the diseased state, physicians should contemplate the use of pharmacological agents or host modulatory therapy.

Tetracyclines are a group of antibiotics produced naturally from certain species of *Streptomyces* or derived semisynthetically. Chlortetracycline (**Figure 1**) is the first tetracycline to be fully

Fig. 1—Polyketide synthesis pathway, tetracycline natural products, and tetracycline semi-synthetic compounds: (I) acetate subunits, (II) polyketide intermediates, and (III) pretetramid.

Figure 1. Polyketide synthesis pathway, tetracycline natural products, and semi-synthetic compounds.

characterized both chemically and clinically. Their advantages were broad-spectrum activity, better tolerated, and less toxic to some individuals. Further modifications of these natural products by means of synthetic reactions and reagents led to the production of the clinically used antibiotic tetracycline compounds—minocycline, doxycycline, and methacycline.

2. Advantages of tetracyclines in periodontitis

Chemically modified tetracycline plays an important role in suppressing the concentration of Gram-negative microorganisms in the subgingival plaque. Concentration in the (GCF) was 5-10 times greater than in serum. Tetracyclines are now recognized to have nonantimicrobial properties that may also be therapeutically advantageous. An ability to promote fibroblast and connective tissue attachment to tooth & other surfaces (**Figure 2**), which is relevant to periodontal regeneration. They have anti-inflammatory properties, particularly in the treatment of certain skin diseases like rosacea, dermatitis herpetiformis and epidermolysis bullosa, disorders that are not thought to have a bacterial etiology and their ability to inhibit host collagenolytic enzymes, an effect that inhibits the connective tissue degradation, including bone resorption [1].

2.1. Matrix metalloproteinases (MMPs)

These are a family of enzymes capable of degrading connective tissue matrix. These enzymes are secreted in latent form by fibroblasts, keratinocytes, macrophages and polymorphoneutrophils.

Figure 2. Role of chemically modified tetracycline in periodontitis.

There are about 28 MMPs. There are about 28 MMPs. All MMPs have similar multi-domain structure (**Figure 3**) Ryan et al. [2]:

1. "Pre" region to target for secretion
2. "Pro" region to maintain latency
3. Catalytic region that contains the active zinc-binding site
4. Proline-rich hinge region, which acts as a zinc-binding site

The majority of MMPs have additional domains, such as hemopexin region or fibronectin-like region, which play a role in recognition and in inhibitor binding.

MMPs can be grouped into six:

- (a) Collagenase
- (b) Gelatinase
- (c) Stromelysins
- (d) Membrane-type MMPs
- (e) Matrilysins
- (f) Other MMPs

Activation and regulation of MMPs (**Figure 4**)

Metal ions

Thiol reagents

Detergents

Organomercurials

Oxidants

Figure 3. Structure of matrix metalloproteinases (MMPs).

Figure 4. Inactive and active MMPs.

Inhibitors of MMPs can be broadly classified as being

- a. Nonsynthetic, e.g., endogenous TIMPs
- b. Synthetic, e.g., collagen peptidomimetics, non-peptidomimetics, bisphosphonates, tetracycline derivatives like chemically modified tetracyclines (CMTs), and subantimicrobial dose doxycycline (SDD)

2.1.1. Host modulatory therapy

It is a treatment concept that aims to reduce tissue destruction and stabilize or even regenerate the periodontium by modifying or downregulating destructive aspects of host response and upregulating protective or regenerative responses [3, 4].

Various host modulatory agents proposed to block pathways responsible for periodontal tissue destruction are:

1. Inhibition of MMPs through CMTs
2. Inhibition of arachidonic acid metabolites
3. Modulation of bone metabolism
4. Regulation of immune and inflammatory responses

2.2. Chemically modified tetracyclines (CMTs)

The unexpected ability of tetracyclines to inhibit the breakdown of the connective tissue and bone by a nonantimicrobial mechanism was first reported over six decades. Based on the thoroughly explored chemistry of tetracyclines, a number of tetracycline analogs can be synthesized with side-chain deletions or, in some cases, moieties added to the parent tetracycline molecule.

Golub and co-workers (1983, 1987) [5] made the observation that collagenase activity was inhibited by tetracycline.

Their study showed that: in severe hypoglycemic rats, there was a shift to Gram-negative microflora in subgingival plaque with more of endotoxin penetration into subepithelial connective tissue leading to stimulation of host cells.

2.2.1. The diabetic rat model

Golub [5] and co-workers modified their experimental diabetes protocol using tetracycline therapy and germ-free rats to determine whether the Gram-negative microflora increased collagenase levels (**Figure 5**).

As a result of these initial studies, Golub et al. proposed:

1. Collagenase action was inhibited by tetracycline.
2. This property of tetracycline can be useful not only for periodontal disease but also for rheumatoid and osteoarthritis (as well as several cutaneous and other diseases) that involve collagen destruction.

Tetracyclines are known to inhibit collagenase and some other, but not all, matrix metalloproteinases or MMPs from a variety of cells:

Figure 5. The diabetic rat model.

Figure 6. Inhibition mechanism of polymorphoneutrophils (PMNs) and fibroblast collagenase.

- Neutrophils
- Macrophages
- Osteoblasts
- Chondrocytes
- A wide range of tissues: skin, gingiva, cornea, cartilage, and rheumatoid synovium

Tetracyclines inhibit PMN but not fibroblast collagenase [6, 7] (Figure 6):

- PMNs are the major sources of collagenase that mediates tissue breakdown instead of fibroblasts.
- During inflammation, collagenolytic activity will be reduced by the use of these drugs but not the collagen turnover.

To identify the site of the anti-collagenase property, Golub and co-workers (1991) [5] synthesized 10 different analogs of tetracyclines known as chemically modified tetracyclines (CMTs 1–10). All 10 analogs lacked antimicrobial efficacy and inhibited collagenase activity, but only 1 did not.

3. Structure of CMT

This modification did not reduce the ability of drug to block the activity of collagenases from a number of tissue sources (PMNs, gingiva, osteoblasts, synovial tissue, lung cancer cells) or

its ability to inhibit bone resorption [8] (Figure 7). Thus, it was concluded that the C-4 moiety had no role in anti-collagenase action of the drug.

When changes were made at C-11 and C-12 position by converting tetracycline to the pyrazole derivative or CMT-5, the collagenase inhibitory activity was lost.

These carbon groups are thought to be the sites for cation binding at physiologic pH 4.8, and all collagenases are known to require the cations, calcium and zinc, for their hydrolytic activity.

Figure 7. Structure of chemically modified tetracyclines (CMTs).

CMT-2 or tetracyclinonitrile was produced by dehydration of the carboxamide residue at carbon 2.

CMT-3 - produced by removing the hydroxyl & methyl groups on carbon 6 & 4.

3.1. Mechanism of action of CMTs

- a. CMTs bind metal ions, particularly Ca^{2+} and Zn^{2+} , which are required by enzymes for their normal activity [9] (Figure 8).
- b. Inhibition of pro-MMPs.
- c. CMTs have also been shown to downregulate expression of MMP-2 and MMP-9 [Golub et al. (1991, 1998)].
- d. Also, CMTs may inhibit the activation of collagenases (MMP-1, MMP-8, and MMP-13)
- e. Inhibit stromelysins (MMP-3, MMP-10, and MMP-11).
- f. Inhibit MT-MMPs.

Other mechanisms that have been proposed include:

- Retards cytokine production.
- Reduced serine proteinase and trypsinogen-2 (Pruzanski et al., 1998; Kirkwood et al., 1999).
- Inhibition of protein glycation.
- Inhibition of non-collagenolytic proteases.
- Inhibit secretion of other collagenolytic enzymes like lysosomal cathepsin.
- Scavenges reactive oxygen species.
- Modulates the osteoclast function and inhibits already active MMPs.

Tetracyclines affect several parameters of osteoclast function (Figure 9).

Figure 8. Mechanism of action of chemically modified tetracyclines (CMTs).

Figure 9. Effect of tetracyclines on osteoclast function.

3.2. Action on *P. gingivalis* and *T. denticola*

- Inhibits Arg- and Lys-gingipain activities and collagenolytic activity of *P. gingivalis*.
- Inhibited trypsin like activity of *T. denticola*.
- CMT-I inhibited serum albumin degradation by *P. gingivalis* and *T. denticola*.
- CMT-1 inhibited the inactivation of $\alpha 1$ proteinase inhibitor by *P. gingivalis*.
- CMT's potential advantages over conventional tetracyclines:
- The recent observations in rats showed that CMT-1 is absorbed after oral administration more rapidly and has a longer serum half-life than tetracycline.

- Their long-term systemic administration does not result in gastrointestinal toxicity.
- No resistance.
- Can be used for prolonged periods.

3.3. Current status of CMTs

- Though not approved for human use by the FDA, preliminary studies using CMT-3 are being investigated on humans with cancer.
- Greenwald et al. recently conducted a synergism study using CMT-1 + flurbiprofen, a standard nonsteroidal anti-inflammatory drug selected primarily because of its reported beneficial effect on bone loss in humans with adult periodontitis and the beagle dog model of periodontal disease.

4. Periostat

It is subantimicrobial dose of doxycycline hyclate (SDD) capsule of 20 mg prescribed for patients with chronic periodontitis twice daily for 3 months, up to a maximum of 9 months of continuous dosing [10]. Indications for periostat are patients who have not responded to non-surgical therapy, patients with generalized recurrent sites of 5 mm or greater pocket depth that bleed on probing, and patients with mild to moderate chronic periodontitis and a high susceptibility to rapid periodontal disease progression. It is the only FDA-approved systemically administered HMT indicated in the treatment of periodontitis [11]. Most patients will have small (i.e., less than 1 mm) additive effect on pocket depth reduction when this systemic approach is added to scaling and root planning. Modulation of host response may be valuable in enhancing effects of antimicrobial agents, such as doxycycline, which are locally released and in periodontitis treatment, especially in smokers.

4.1. Mechanism

The rationale for using SDD is based on the concept of host modulation; that is, it downregulates the activity of destructive responses such as MMP activity and upregulates protective responses as it promotes osteoblastic activity leading to new bone formation by upregulating collagen production.

4.2. Indications

- a. Indicated in the management of chronic periodontitis
- b. Can be used in patients with Ag periodontitis who are treated nonsurgically
- c. Can also be used as an adjunct to periodontal surgery

- d. May also be beneficial in cases that are refractory to treatment, as well as in patients with risk factors such as smoking or diabetes, in whom the treatment response might be limited

4.3. Contraindications

SDD should not be used in conditions such as gingivitis and periodontal abscess or when an antibiotic is indicated. The other contraindication of its use is in allergy to tetracycline. Patients taking oral contraceptives may have reduced protection from pregnancy with this therapy.

Host modulation therapy is an emerging treatment concept and can be used in susceptible, high-risk patients in whom a prolonged and excessive host response to microorganisms promotes activity of MMPs and osteoclast. Clinical trials have demonstrated a clear treatment benefit when it is used in combination with phase I therapy. The further development of these agents will help clinicians to treat specific aspects of periodontal diseases by reducing inflammation and inhibiting destructive processes in tissues, which will result in enhanced periodontal stability after conventional periodontal treatment such as scaling root planning (SRP) and surgery.

Ramamurthy et al. (2002) tested doxycycline and five different CMTs to prevent MMP-dependent periodontal tissue breakdown in an adult rat model. CMTs were administered orally at conc. 2 mg/day for 7 days, and gingival biopsies were taken to assess cytokines TNF, IL-1, and MMP-2 and MMP-9. All tetracycline inhibited periodontal breakdown in the following order of efficacy: CMT, 8 > 1 > 3 > doxy > 4 > 7.

Long-term (i.e., 9–18 months) administration of SDD does not result in emergence of resistant organism or alteration of subgingival microflora. Various long term studies have been conducted.

Doxycycline 20 mg bid was used in 51 patients with active periodontitis based on pocket depth and increased collagenase at multiple exams showed no clinical attachment loss over 36 months.

Twenty subjects ≤ 45 year old patients with severe generalized periodontitis patients and showing >30% of sites with CAL ≥ 5 mm were given subantimicrobial dose of doxycycline for 6 months. The result showed less clinical attachment loss, probing depth. Gingival index and bleeding on probing were not significant when compared to placebo.

Doxy 20-mg bid for month along with scaling and root planning was done in 208 chronic periodontitis subjects. The patients showed improvements in clinical attachments level and probing depth ≥ 4 mm.

Used 20 mg bid SDD for 9 months + scaling and root planning in 190 adult periodontitis patients, there was a significant improvements in CAL (≥ 2 mm) PD and BOP when compared to placebo group receiving only SRP.

Author details

Anshul Sawhney

Address all correspondence to: dranshul1986@yahoo.co.in

Department of Periodontics, Uttar Pradesh University of Medical Sciences, Saifai, India

References

- [1] Golub LM, Sorsa T. Host modulation with tetracyclines and their chemically modified analogs. *Current Opinion in Dentistry*. 1992;**2**:80
- [2] Ryan ME, Preshaw PM. Host modulation. In: Newman, Takei, Carranza, editors. *Clinical Periodontology*. 10th ed. WB Saunders; 2007. pp. 275-282
- [3] Genco RJ. Host response in periodontal disease: Current concepts. *Journal of Periodontology*. 1992;**63**:338
- [4] Kornmann KS. Host modulation as a therapeutic strategy in the treatment of periodontal disease. *Clinical Infectious Diseases*. 1999;**28**:520
- [5] Golub LM, Mc Namara TF, Angelo GD, Greenwald RA, Ramamurthy NS. A non-antibacterial chemically-modified tetracycline inhibits mammalian collagenase activity. *Journal of Dental Research*. 1987;**66**(8):1310-1314
- [6] Ryan ME, Kinney J, Kim Amy S, Giannobile WV. The host modulatory approach. *Dental Clinics of North America*. 2005;**49**:624-635
- [7] Salve GE, Lang NP. Host response modulation in the management of periodontal disease. *Journal of Clinical Periodontology*. 2005;**32**(suppl. 6):108-129
- [8] Devulapalli SN, Sahitya S, Srinivas M, Vasavi K. Chemically modified tetracyclines: The novel host modulating agents. *Journal of Indian Society of Periodontology*. Jul-Aug 2015;**19**(4)
- [9] Wang Y, Morlandt AB, Xu X, Carnes DL, Chen Z, Steffenson B. Tetracycline at subcytotoxic levels inhibits matrix metalloproteinase-2 and -9 but does not remove the smear layer. *Journal of Periodontology*. 2005;**76**:1129-1139
- [10] Greenstein G, Lamster I. Efficacy of subantimicrobial dosing with doxycycline. *Journal of the American Dental Association (Chicago, IL)*. 2001;**132**(4):457-466
- [11] Choi DH, Moon IS, Paik JW, Kim YS, Choi SH, Kim CK. Effects of sub-antimicrobial dose doxycycline therapy on crevicular fluid MMP-8, and gingival tissue MMP-9, TIMP-1 and IL-6 Levels in Chronic Periodontitis. *Journal of Periodontal Research*. 2004;**39**:20-26