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CHEMICALLY MODIFIED TETRACYCLINES: A REVIEW

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ABSTRACT

Periodontitis is a microbial infectious disease that causes loss of supporting tooth structures. Host-microbial interaction can aggravate the destruction of periodontium. Hence host modulatory agents such as tetracycline and its derivatives like Chemically Modified Tetracyclines (CMTs) have been proposed. CMTs lack antimicrobial action but have anti-collagenase activity. CMTs reduce the MMP production and also reduce the amount of proinflammatory cytokines produced. They do not cause antibiotic resistance unlike tetracycline. Hence, CMTs are considered to be one of the potent host modulation agents that can be used for treatment of periodontitis.

KEYWORDS: Periodontitis, MMPs, Tetracyclines.

INTRODUCTION

Periodontitis is defined as an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or group of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession or both. Various therapeutic options include mechanical, surgical debridement, administration of systemic, local drugs or combination of all. Host Modulation 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. NSAIDs, Bisphosphonates, Tetracyclines

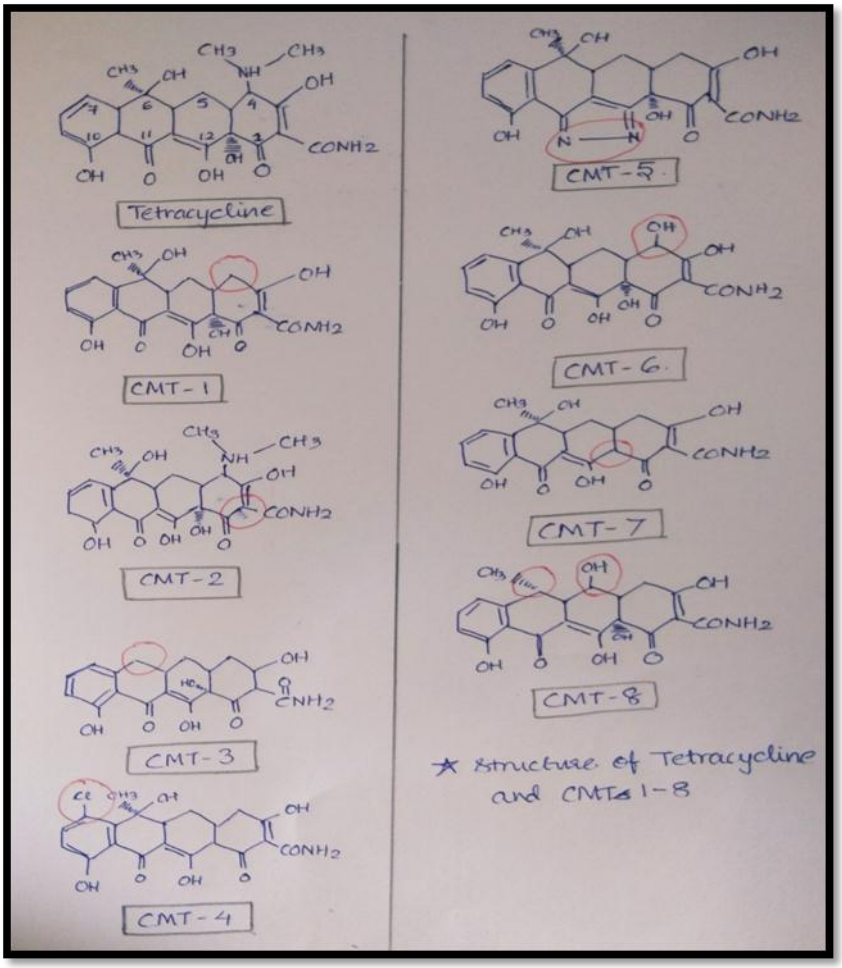
etc are used as host modulating agents. Among these, Tetracyclines are most commonly used. They have an ability to inhibit collagenase as well as some matrix metalloproteinase activity, thus reducing periodontal destruction. This observation was done by Golub and his co-workers in 1987 on a diabetic rat model.[1]

History

Tetracycline was first discovered by Benjamin Minge Duggar. By the mid-1950s, three tetracyclines were used clinically, their chemical names became in order of their discovery, chlortetracycline (Aureomycin), oxytetracycline (Terramycin) and Tetracycline. Further modifications of these natural products were done by means of synthetic reactions and reagents, which led to the production of the clinically used antibiotic tetracycline compounds like minocycline, doxycycline, and methacycline. Mitscherin 1991 had thoroughly explored chemistry of the tetracyclines, where he concluded that a number of tetracycline analogs can be synthesized with side-chain deletions or, in some cases, moieties added to the parent tetracycline molecule.[2] To identify the site of the anti-collagenase property of Tetracycline, Golub and co-workers[1] synthesized 10 different analogs of tetracyclines known as chemically modified tetracyclines (CMTs1–10). The analogs had anti- collagenase activity but lacked antimicrobial efficacy.

Structure

Tetracycline consists of four rings and contains many groups such as alkyl, hydroxyl and amine on the upper and lower sides of the molecule. Tetracyclines with antibiotic activity have a dimethylamine group at C4 in the ring A. Addition or removal of the dimethylamino group from C4 gives several chemically modified tetracyclines.



Removal of the dimethylamino group from the carbon-4 position of the "A" ring, resulting in the CMT called 4-de-dimethylaminotetracycline (CMT-1). CMT-2 or tetracyclinonitrile was produced by dehydration of the carboxamide residue at carbon 2 of the tetracycline molecule. CMT-3 (6-deoxy 6-demethyl 4-de-dimethylaminotetracycline) was produced by removing the hydroxyl and methyl groups on carbon 6 from the CMT-1, and CMT-4 (7-chloro 4-de-dimethylaminotetracycline) was produced by removal of the dimethylamino group from carbon 4 of chlortetracycline. When the carbonyl oxygen at carbon 11 and the hydroxyl group at carbon 12 was removed by converting tetracycline to the pyrazole derivative or CMT-5, as described by Valcavi et al., the collagenase- the inhibitory activity of the molecule was lost.[3] CMT-5 was also ineffective against collagenase extracted from diabetic rat skin.[4] This strongly suggests that these side chains on the tetracycline molecule, which are known to be major (but not the only) metal-ion binding sites on the drug, constitute the site of the anti-collagenase activity.[5]

Based on these findings, a few other modifications were made to the core tetracycline structures by addition or deletion of functional groups to produce CMT-6 (4-dedimethyl amino 4- hydroxytetracycline), CMT-7 (12α-Deoxy, 4-dedimethylamino tetracycline), CMT-8 (4-dedimethylamino doxycycline), CMT-9 (12α,4α-Anhydro, 4-dedimethylamino-tetracycline), and CMT-10 (7-Dimethylamino, 4-dedimethylamino-tetracycline), which is derived from minocycline.

MECHANISM OF ACTION

CMTs act via

1. Inhibition of MMPs
2. Inhibition of proinflammatory cytokines
3. Inducible nitric oxide synthase (iNOS)
4. Inhibition of bone resorption
5. Enhancement of the attachment of fibroblasts and connective tissues to the tooth surface

Inhibition of MMPs

The matrix metalloproteinases (MMPs) are zinc and calcium dependent endopeptidases secreted by cells like PMNs, macrophages, fibroblasts, epithelial cells, osteoblasts and osteoclasts etc. MMPs destroy extracellular matrix components like collagen, gelatin, laminin, fibronectin and proteoglycans. Increased activity of MMPs is seen in chronic inflammatory conditions including periodontitis, rheumatoid arthritis and cancer.

MMPs can be inhibited

(a) With the help of Ca2+ and Zn2+-binding sites

(b) By inhibition of reactive oxygen species-mediated activation of pro-MMPs,

(c) By proteolysis of pro-MMPs into enzymatically inactive fragments, protection of α-1 proteinase inhibitor from MMPs, and

(d) By reduction in the activity of serine proteinases.

Neutrophils are the major source of collagenases. They mediate the connective tissue breakdown whereas the fibroblasts contribute collagenase that is required for connective tissue remodeling in normal gingiva. In CMTs, anti-collagenase activity is present on carbon no. 11 & 12 of the four ringed structure. The CMTs are specific against the collagenase that is produced from neutrophils but not from the fibroblasts. This helps in the reduction of pathologic concentrations of collagenases enzyme without affecting the normal collagen

turnover required to maintain the tissue integrity. The CMT-3 is specifically active toward MMPs because of its pleiotropic action. It also exerts an inhibitory effect on MMPs in micromolar concentrations by reduction in trypsinogen-2 and inducible nitric oxide (iNOS) production.[6] A study comparing six different CMTs in inhibition of MMPs showed that the CMT-8 was most effective inhibitor of periodontal breakdown. Tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), interleukin-.6 (IL-6) and MMPs were inhibited by CMT-8,-1, -3, -4, -7 and doxycycline in descending order.[7]

Chemically modified tetracycline-3 was found to inhibit matrix metalloproteinase expression activity in human colon and breast cancer cells, in addition to cytostatic effects on human renal and prostate cancer. In vivo administration of this novel compound to rats with prostate cancer inhibited tumor growth and metastasis. The National Cancer Institute in collaboration with Dezube et al tested CMT -3 in phase I and phase II clinical trials. In this study, chemically modified tetracycline-3 was administered once a day to patients with AIDS-related Kaposi sarcoma, who exhibited a 44% response rate, reflecting a decrease of angiogenic lesions, which was associated with a suppression of MMP-2 levels in the circulation. In a larger phase II clinical trial, group of patients with Kaposi sarcoma, who received a daily oral dose of 50 mg of chemically modified tetracycline-3, showed a significant reduction of angiogenic lesions, whereas the individuals who were administered the higher dose (100 mg once a day) did not. Significant reductions in plasma levels MMP-2 and MMP-9 were observed, and the most common adverse events were photosensitivity and rash, which could be quite severe.[8]

b) Inhibition of proinflammatory mediators

Pro-inflammatory mediators like IL‑1, IL‑6, IL‑8, TNF-α and PGE 2 can cause destruction of periodontal supporting tissues. CMTs are found to have inhibitory effects on these pro-inflammatory mediators. It was found that CMT‑3 inhibits COX‑2‑mediated PGE‑2 production, intracellular accumulation and synthesis of TNF-α in activated mast cells, IL‑8 and protein kinase – C production.[9,10]

In an ex vivo study, human whole blood model was stimulated with P.gingivalis is LPS. The efficacy of Doxycycline and CMT‑3 was investigated in suppressing the production of proinflammatory mediators and MMPs. It was seen that there was a significant reduction in the secretion of proinflammatory cytokines but the levels of MMPs were not affected.[11]

3. Inhibition of inducible nitric oxide synthase (i-NOS)

Nitric oxide is one of the activators of MMPs. The end product of NO, peroxynitrite is highly cytotoxic, inhibits collagen and proteoglycan synthesis and upregulates the MMP expression, thus eventually causing tissue and bone destruction. CMT-3 have found to exhibit an inhibitory action on inducible nitric oxide synthase activity. Inhibition of iNOS production causes reduction in the peroxynitrite levels, thus preventing denatuartion of proteins. CMT-3 and CMT-8 are most effective and have maximum inhibitory effect on the iNOS whereas CMT-1 and-2 have an intermediary effect and CMT-5 was not effective.[12]

4. Inhibition of bone resorption

CMT-3 and CMT-8 has found to retard osteoclastic bone resorption and enhance bone formation and wound healing. It also inhibits proteinases produced by the action of microbes. CMT-1, -3, -6, -7 and -8 were potent inhibitors of osteoblastic collagenase in culture. CMTs reduces the bone resorption and promote bone formation by different mechanisms like reduction in number of osteoclasts by inhibiting their development and inducing apoptosis, by increasing the size of clear zone, by decreasing the production of osteoclastic enzymes, inhibits osteoclastogenesis, increasing intracellular calcium levels, inhibiting osteoclasts, collagenase production and also decreases acid production, thereby retarding bone resorption, thus preventing the destructive progression of periodontal disease. Because of this pleiotropic action, it provides significant therapeutic potential for the treatment of periodontitis.

5. Action on P.gingivalis and T. denticola

CMTs Inhibits Arg- & Lys- gingipain activities & Collagenolytic activity of P.gingivalis. It also inhibited trypsin like activity of T.denticola.CMT-I inhibited serum albumin degradation by P.gingivalis & T.denticola.CMT-1 inhibited the inactivation of α1 proteinase inhibitor by P.gingivalis.

Comparison of Chemically Modified Tetracyclines

CMTs have distinct pharmacological 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. CMT-3 has long serum half-life that is 2.1 to 11.0 times longer than any other tetracycline compounds. 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.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. 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.[13]

CMT-3 is the only chemically modified, non-antimicrobial analogue of tetracycline clinically tested in humans. 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). CMT-3 has shown superior pharmacokinetics including a long serum half-life after oral administration and highly lipophilic.[12]

USES

CMTs can be used in the management of various medical conditions associated with abnormal concentration and activity of collagenase. Some of these conditions are as follows:

1) Rheumatoid arthritis

Rheumatoid arthritis is a chronic inflammatory disease primarily causing bone, joint or tissue destruction. The PGE-2 levels increase the local blood flow and potentiates the action of mediators such as bradykinin. This affects the cellular functions causing activation of MMPs, induction of apoptosis, inhibition of chondrocytic growth, activation of osteoclastic bone resorption, upregulation of IL‑1transcription factor and cAMP levels. Both in vitro and in vivo studies have demonstrated the beneficial role of CMT‑1 in suppressing the collagenase activity in the cultured synovial tissue. However, a combination therapy using CMT‑1 and flurbiprofen produced a greater suppression of the clinical inflammation along with radiographic improvement of the joint condition. This was due to the synergistic effects of anti‑inflammatory action of flurbiprofen along with anticollagenase action of CMT‑1.[14]

CMT‑3 and ‑ 8 have also shown inhibitory effect on COX‑2‑induced PGE2 production. Additional animal and human trials are needed to evaluate the efficacy of these agents in treatment of arthritis.

2) Diabetes mellitus

Experiments in diabetic rats showed that daily oral administration of CMT for 21‑37 days reduced levels of pathologically excessive collagenase in gingival tissues and skin. The CMTs also increased the skin collagen production as revealed by increased concentrations of

hydroxyproline. There was increased osteoblastic activity and bone formation.[15] In vitro and in vivo studies in rats with both the types of diabetes showed that the CMTs inhibited MMP activity, enzyme expression and alveolar bone loss. Administration of CMT‑8 in type II diabetic rats with nephropathy or retinopathy showed a reduction in the incidence of cataract development, proteinuria, and tooth loss. The results were better with CMTs as compared to the commercial tetracyclines.[16]

3) Tumor metastasis

Recently, it has been reported that the CMTs are effective in treating metastasis which accounts for more than 90% of cancer mortality. Excessive production of matrix‑degrading MMPs and serine proteinase enzymes generated by tumor cells creates an imbalance between the destructive enzymes and their inhibitors. Tetracyclines, especially the minocycline and CMTs have been investigated for their antimetastatic actions in the tumors of prostate, breast and melanomas. They not only inhibit the invasive potential and MMP activity, but also cell proliferation by inducing cell cycle arrest and apoptosis.[17] The CMTs kill tumor cells by generation of hydroxyl free radicals which permeate and depolarize mitochondria. They also activate caspase‑mediated apoptosis and reduce the rate of angiogenesis. Inhibition of the type IV collagenase prevents the tumor cells from invading the basement membrane barriers and hence metastasis.[19] In a phase II trial of CMT‑3 in the treatment of Kaposi'ssarcoma in HIV patients, a significant decrease in serum MMP-2 and MMP-9 levels was seen. It was suggested that high doses of CMT‑3 (50–150 mgqd) may be beneficial in the Kaposi's sarcoma patients who did not respond to highly active antiretroviral therapy alone. This may be attributed to its ability to inhibit neutrophil elastase.[8]

4) Role of CMTs in Wound Healing

CMTs inhibit the release of pro-inflammatory mediators. CMTs are designed to be more potent inhibitors of proinflammatory mediators and can increase levels of anti-inflammatory mediators such as IL-10. CMTs also increase integrin expression on endothelial cells in inflammation, counteract the effects of transforming growth Factor-β induced expression of MMPs, enhance phagocytosis by increased expression of FcγRIII, and stimulate fibroblasts to produce protease inhibitors like tissue inhibitors of matrix metalloproteases (TIMPs).[18]

5) In Periodontal Diseases

CMTs have anti collagenolytic action because of which can be given for management of periodontal disease. And also it has found to have an inhibitory action against P.gingivalis and T. denticola**.**

6) Other uses

CMT‑3 has been shown to have antifungal properties. Lower oral doses of CMT‑3 are effective in decreasing the severity of acne. They have also been used in the treatment of life threatening conditions like epidermolysis bullosa and acute respiratory distress syndrome associated with excessive collagenase activity.

Current Status of Cmts

CMTs have not yet been approved for human use by the FDA, although the National Cancer Institute has recently initiated preliminary studies, using CMT-3, on humans with cancer. More recent studies have demonstrated the therapeutic potential of TCs' anti-MMP activity in in vivo and cell culture models of cancer invasion, metastasis, and angiogenesis (Masumori et al, Lokeshwar et al).[19,17]

CONCLUSION

The CMTs are still under research as they lack approval due to excessive suppression of MMPs which may alter the normal turnover rate of collagen. Further research is needed on CMTs which may be useful in suppressing the extracellular MMPs and the intracellular targets are warranted. The CMT-3 has been shown to be the most promising agent among all CMTs. CMTs will likely emerge as drugs that have beneficial effects in a variety of disease status because of their host modulating capabilities.

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