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Chemically modified tetracyclines an emerging host modulator in chronic periodontitis patients: A randomized, double-blind, placebo-controlled, clinical trial

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ABSTRACT

Although periodontal diseases are caused by some of the specific pathogens, most of the tissue damage is caused by the host reaction to disease and not actually by the infections. Therefore, host modulatory therapy (HMT) has advanced benefit for the treatment of periodontitis, which works basically by reducing tissue destruction and regeneration in periodontium by altering the critical aspects of host response regulation and up regulating defensive regenerative responses. The present study was conducted with the goal to test an innovative therapeutic option using chemically modified tetracycline in patients affected with generalized, moderate and severe chronic periodontitis. We assumed that CMT might have the potential to provoke an assessable clinical result and pharmacologically impede the level inflammatory flow. CMT (incyclinide) treated group had significantly higher CAL (clinical attachment) values than Placebo Control suggesting an improved CAL in CMT treatment. Host modulation therapy width incyclinide can be as an adjunct to conventional nonsurgical therapies without antimicrobial resistance. Progress was noticed in the clinical parameters but not the serum CRP level in our study establishing the role of CMTs in controlling chronic periodontitis. Also CMT treatment indicates its role in anti-inflammatory process as it inhibited IL-12 and TNF alpha but IL-10 level was not affected. However, more randomized placebo-controlled clinical trials with large sample size are required in order to authenticate the usage of CMTs in chronic periodontitis treatment. Based on this understanding, exploration of the novel, low-cost synthetic inhibitors that can be used as potential therapeutic agents, has been tested.

1. Introduction

Chronic periodontitis involves inflammation of the periodontal tissues, adjacent connective tissues and teeth alveolar bone [1], eventually leading to substantial dental plaque accumulation, bone loss, and tissue destruction. An exaggerated immune response induced by different inflammatory agents such as cytokines, arachidonic acid (AA) metabolites and enzymes [2] is usually observed in this condition. Specific pathogenic bacteria are the main causal agents of periodontal diseases. However, most of the tissue damage is caused by the host reaction to disease and not by the infections. The pathological manifestations of periodontitis are due to the matrix metalloproteinases (MMPs) produced by infiltrating and resident cells of the periodontium. An imbalance produced by the activated MMPs and their endogenous inhibitors leads to the breakdown of the extracellular matrix during periodontitis [3].

Host modulatory therapy (HMT) has evolved for profiting the treatment of periodontitis, basically by targeting to reduce tissue destruction and regeneration of the periodontium by altering the critical aspects of the host response regulation and up-regulating the defensive or regenerative responses [4]. There is considerable attenuation in inflammatory processes with HMT and marked improvement in wound prognosis manifested by periodontal resoluteness. No damage is rendered whatsoever to the host

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defense responses mechanisms. Different types of HMT have been tried and tested as part of periodontal therapy plan, which involves non-steroidal anti-inflammatory drugs, tetracyclines, and bisphosphonates [5].

Notably, biologically active tetracyclines have been chemically modified (Chemically modified tetracycline, CMT) and considered as potential host modulating agents. Due to chemical modification, CMT lacks the antimicrobial activity, due to which chances of development of antibiotic resistant microbial flora diminishes in long-term therapy. Other advantages of CMT include the absence of gastrointestinal toxicity with long term systemic administration and increased plasma concentrations even with less frequent drug administration [6]. Previous studies reported the immense therapeutic value of CMT that also included the inhibition of neutrophil chemotaxis; improved fibroblast add-on to the root surface, anti-inflammatory property due to the inhibition of prostaglandin synthesis, inhibition of bone resorption and improvement of collagen synthesis [7].

Modifications in the core structure of the tetracyclines by adding or removing functional group have resulted in more than 8 CMTs available for current use [6,8,9]. Among them, CMT-1, CMT-3, and CMT-8 have been tested for medical applications and it has been found that CMT-3 and CMT-8 are potent inhibitors of tumor metastasis and synoviocyte invasion in animal models affected with arthritis [8,9].

With this background knowledge about CMT and its scope of being used as HMT, a randomized, double-blind, controlled clinical trial was designed and executed with a goal to test an innovative therapeutic option using CMT for patients affected with generalized moderate and severe chronic periodontitis. Our hypothesis was that CMT has the potential to provoke a measurable clinical result and pharmacologically attenuate the inflammatory response. Clinical outcomes of active versus placebo therapies were measured that included bleeding on probing (BOP), clinical attachment level (CAL), and the percentage of sites with pocket depths (PDs).

C-reactive protein (CRP), a marker of systemic inflammation, is an extremely sensitive but nonspecific acute-phase marker for inflammation. A positive relationship between CRP and periodontitis has been recognized in the past, reflected by a higher serum CRP levels in patients with periodontal disease. Serum CRP quantification assay was therefore performed considering the fact that CRP is a potential biomarker for periodontitis and systemic disease [10–12].

2. Materials and methods

We initiated a double-blind parallel placebo-controlled randomized controlled trial (RCT), which was ethically approved by the Institutional Ethics Committee at KVG Dental College and Hospital, Sullia, Karnataka, India. All the patients reporting to Department of Oral Medicine and Radiology were explained about the nature of studies and outcome of the results, following which the patients gave their informed consent and were enrolled in the study.

2.1. Sample size and exclusion criteria

A total of 56 patients were calculated as minimum required sample size for each group; however, 65 patients in each group were enrolled keeping in mind any unexpected fall in numbers from the finally enrolled. This study was driven by 90% to detect a mean pocket depth (PD) difference of 1.0 mm after clinical management with the presumption that a 30% within-group change may be observed in the primary outcomes PD and clinical attachment level (CAL).

We classified the patients into two groups; a control group receiving two placebo capsules two times a day for three weeks and study group receiving two incyclinide (Commercially available CMT; CollaGenex, Newtown, PA, US) capsules two times a day for three weeks. Smokers, patients with allergies, systemic illness, pregnant women, patients with history of any drug intake, and patients having undergone periodontal treatment in the past 6 months were excluded from the study. Patients with moderate ($\geq$2 interproximal sites with CAL of $\geq$4 mm or $\geq$2 interproximal sites with PD of $\geq$5 mm) and severe chronic periodontitis ($\geq$2 interproximal sites with CAL of $\geq$6 mm and $\geq$1 interproximal site with PD of $\geq$5 mm) were selected for the study.

Since the study design was double-blinded, all the details of the patients and their classification were sequestered by randomization according to CONSORT (consolidated standards for reporting of trials) 2010 guidelines. This ensured complete unawareness on part of the allocator and the examiner regarding the group of the patient. Thus, the two groups (the study group SG and the control group CG) of 65 patients each were monitored by the data monitoring group and patient allocation was done by random key generator (GraphPad QuickCalcs, GraphPad Software Inc., San Diego, CA, USA) by providing a number to track the patients' group (either SG or CG).

2.2. Intervention

The periodontal parameters including Plaque Index (PI), gingival index (GI), sulcus bleeding index (SBI), PD, and CAL were registered before starting treatment. Primary parameters included serum CRP, PD, CAL, whereas, secondary parameters included PI, oral hygiene index simplified, GI, and SBI. These primary and secondary outcome measures were recorded until 12 weeks in both the treatment groups. UNC 15 probe was used to measure PD and CAL. Mesiobuccal, buccal, distobuccal, and palatal or lingual sites were identified to measure the periodontal parameters and the mean average was calculated. Serum CRP levels of the patients were recorded at each visit. Initial therapy includes full mouth Scaling and Root planning (SRP) which was performed by a periodontist after local anesthesia either by hand or ultrasonic instrumentation and instructed to follow oral hygienic instructions with compliance.

Thereafter, the drug incyclinide (Sub-antimicrobial dose SDD) 20 mg twice daily was dispensed by the data monitoring group wherein the SG received CMT and the CG received placebo in similar packets. Periodontal parameters were recorded for subsequent visits.

2.3. Laboratory tests

10 ml vein-punctured blood was collected in a vacutainer and centrifuged for 10 min to collect the serum and was stored at −70 °C until analysis. CRP was assayed by using CRP turbilatex kit, a latex particle-enhanced turbidometric immunoassay. Agglutination was detected and quantification of CRP was done based on absorbance using a calibration curve.

2.4. Assay for anti-inflammatory ability of CMT

2.4.1. Periodontal cell line and reagents

The periodontal ligament stem cells (PDLSCs), which is known to secret various interleukins proinflammatory cytokines (IL-1β, IL-6, and IL-8) was used in this study [13] and obtained from the University cell culture collection. The cells were maintained in complete culture medium. RPMI-1640, tissue culture medium supplemented with glutamine (2 mM), antibiotics (penicillin and