ZINC PLUS OCTENIDINE: A NEW FORMULATION FOR TREATING PERIODONTAL PATHOGENS. A SINGLE BLIND STUDY

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Periodontal treatment has the aim to reduce oral infection, and prevent the progression of the disease. The potential benefits of new chemical devices for periodontal therapy, include improved patient compliance, an easier access to periodontal pocket and a lower dosage of antimicrobial agent. The objective of this study was to explore the efficacy of a chemical device containing zinc and octenidine in the treatment of chronic periodontitis in adult patients. Ten patients with a diagnosis of chronic periodontitis (20 localized chronic periodontitis sites) in the age group of 35 to 55 were selected. None of these patients received any surgical or non-surgical periodontal therapy and demonstrated radiographic evidence of moderate bone loss. The chemical device zinc plus octenedine was used by each patient after daily oral hygiene. Microbial analysis were analyzed at baseline and on the 15th day. After the treatment, a remarkable decrease in bacteria amount, both for some species and for the total count was observed in the study group. Specifically T. forsythia and T. denticola were eradicated whereas Total Bacteria Loading and Fusobacterium Nucleatum showed a reduction of 38% and 55%, respectively. Our study demonstrated the efficacy of the new chemical device containing zinc and octenidine in a sustained release drug delivery system in the management of moderate to severe chronic periodontitis.

The term periodontal disease (PD) is generally used to describe diseases affecting the gums and tooth support tissues, causing damage to the connective tissue and alveolar bone (1). Specific bacteria of the biofilm formed by plaque cause PD. The bacteria leak into the periodontal ligament space, causing anaerobic infection creating a cascade of events, which end with the production of inflammatory mediators and bacterial metabolites (1). PD affects about the 50% of adults, or more, while the severe form periodontitis affects around 10% (range: 5-20%) of adults and moderate periodontitis around 30%. In elderly people,

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the prevalence of PD is estimated about 70-90% of individual's aged 60-74 years (1).

Periodontal therapy is important to reduce local inflammation and bacteremias. Recently it has been stated that periodontal disease appear to increase the risk of cardiovascular disease, pulmonary disease, preterm and low birth weight (2).

The oral microbiota is constituted by a large number of bacteria species that form a biofilm. The biofilm includes both saprophites and potentially pathogenic species. Periodontal treatment has the aim to reduce oral infection, and prevent the progression of the disease[3]. Periodontal non-surgical therapy with scaling and root planing associated with a good level of domestic oral hygiene can prevent the onset of the disease and allow for proper maintenance of oral health (3, 4).

Good oral hygiene has the aim to control bacterial plaque, but when the patient's attention to oral hygiene decreases, it is possible to experience a recurrence of the disease. In addition, periodontal disease presents a characteristic trend in pousse with periods of remission and exacerbation of the disease. Support periodontal therapy is efficacy, but a better microbiological control of oral microbiota can be reached by the administration of topical antimicrobials in association.

The advantages of topical therapy involve the insertion of antimicrobials agents directly into the periodontal pocket, minimizing the adverse effects related to systemic therapy (4). The potential benefits of local drug delivery include improved patient compliance, an easier access to periodontal pocket and a lower dosage of the antimicrobial agent. The most commonly used methods for local drug delivery are topical drugs in gel form (5). The antimicrobial agents in the local drug delivery devices include tetracycline, ofloxacin, clindamycin, chlorhexidine, benzalkonium etc. (4)- and have been used either alone or as adjunct wich support periodontal therapy. The local antimicrobials are administered directly into the periodontal pocket and their effectiveness is related to their bactericidal activity and to the subsequent reduction of gingival inflammation (6).

Octenidine has been extensively characterized and poses no risk in terms of acute, subchronic and chronic toxicity, irritation and allergenicity. It exhibits a broad spectrum of antimicrobial efficacy against Gram-positive and Gram-negative bacteria and fungi and the efficacy is not adversely affected by interfering substances like blood and mucous. On skin, it shows a strong residual effect which can still be observed 24 h after application (7). Octenidine belongs to the bispyridinamines (8), a different class of chemicals with respect to chlorexidine and benzalkonium and due to its excellent antimicrobial activity and tissue compatibility, has been chosen as a component of the new gel formulation.

Zinc (Zn) has been advocated since stimulates epithelialization of wounds and exhibits a number of beneficial antimicrobial effects. In *in vitro* studies, Zn inhibits a range of microbial enzyme systems, including a range of glycolytic enzymes in streptococci, with concomitant increased sensitivity of the organisms to acid (9). In two typical oral anaerobes, *Fusobacterium nucleatum* and *Prevotella intermedia*, a range of enzymatic processes are inhibited by Zn, including catabolism of amino acids, sugars and peptides, as well as oxidative metabolism (10). Zn can also deliver beneficial effects for bad breath: reducing breath concentrations of volatile sulphur compounds (VSCs) associated with halitosis, (11, 12) as well as have direct and indirect inhibitory effects on anaerobe mediated- VSC production.

The aim of this study was to investigate the efficacy a new hydroxypropyl-cellulose gel, containg zinc in association with octenidine, acting as a drug delivery system in the management of moderate to severe chronic periodontitis.

MATERIALS AND METHODS

Ten patients were randomly selected with a diagnosis of chronic periodontitis. Patients who qualified for the study were in the age group of 35-55 and did not receive any surgical or non-surgical periodontal therapy. The patients were excluded from the study if they meet any of the following criteria: (1) pregnancy; (2) a history of taking antibiotics or using anti bacterial mouth rinses for the past 6 months; (3) has teeth with furcation involvement; (4) patients with a history of smoking, and drug or alcohol abuse.

The subjects that volunteered to participate in the

study, signed consent forms and followed a detailed verbal description of the procedure.

Clinical methods

Twenty sites were selected from the 10 patients. Each selected site was subjected to microbial analysis. At the first visit, after recording the clinical parameters at each site in selected patients at baseline, microbiological samples were collected from pre-selected sites. At 15 days following the first visit, the patients were checked for any adverse reaction. Verbal instructions were given to avoid manipulating the study teeth.

At each visit (Baseline and 15th), the clinical parameters were assessed. A single trained and calibrated examiner who was blinded to the treatment method obtained all readings. Microbiological samples were collected from the sites of the patients at baseline and at the 15th day.

Clinical parameters were assessed sequentially and the pocket depth, the distance between the base of the pocket and gingival margin, measured. For the collection of subgingival samples, the sites were isolated using cotton rolls. Sterile absorbable paper points (size 60) were used for the collection of subgengival samples and were immediately transferred to microbiological laboratory for processing. The microorganisms processed were the three bacterial species more involved in periodontitis that constitute the red complex group: *Porphyromonas gingivalis, Tannerella forsythia*, and *Treponema denticola.*

Table I. *Bacterial count using PCR at T0 (beginning) and T1 (end of treatment).*

	Number of cases	Minimum	Maximum
Total Becterial Loading T0	10	2796	53080
Total Becterial Loading T1	10	1813	20422
Fusobacterium Nucleatum T0	10	0	1320
Fusobacterium Nucleatum T1	10	0	593
Treponema Denticola T0	10	0	149
Treponema Denticola T1	10	0	0
Tannerella Forsythia T0	10	0	152
Tannerella Forsythia T1	10	0	0

Several methods have been used for microbiological testing in periodontitis (13). However, many techniques have not been fully accepted due to low sensitivity or specificity, moreover sometimes they are slow, expensive and laborious. In this study we have used the (LAB-test®) developed by the LAB corporation (Ferrara, Italy), which is rapid and sensitive, allowing to detect and quantify the three bacterial species more involved in periodontitis that constituted the red complex group: *Porphyromonas gingivalis, Tannerella forsythia*, and *Treponema denticola.* Both *P. gingivalis* and *T. denticola* occur concomitantly with the clinical signs of periodontal destruction (14-22). They appear closely 'linked' topologically in the developing biofilm and are considered the first pathogens involved in the clinical destruction of periodontal tissues (23-28).

Real-Time Polymerase Chain Reaction

Primers and probes oligonucleotides have been designed based on 16S rRNA gene sequences of the Human Oral Microbiome Database (HOMD 16S rRNA RefSeq Version 10.1) counting 845 entries. All the sequences were aligned in order to find either consensus sequences. For each sample, two real-time polymerase chain reaction (PCR) runs were performed. The first reaction quantified the total amount of bacteria using two degenerate primers and a single probe, matching a highly conserved sequence of the 16S ribosomal RNA gene. The second reaction detected and quantified the three red complex bacteria in a multiplex PCR. The reaction included a total of six primers and three probes that were highly specific for each specie. Oligonucleotide concentrations and PCR conditions were optimized to ensure sensitivity, specificity and no inhibitions in case of unbalanced target amounts. Absolute quantification assays were performed by using the Applied Biosystems 7500 Sequence Detection System. The amplification profile was initiated by a 10 min incubation period at 95°C to activate polymerase, followed by a two-step amplification of 15 s at 95°C and 60 s at 57°C for 40 cycles. All the experiments were performed including nontemplate controls to exclude reagents contamination (29-32).

Plasmids containing synthetic DNA target sequences (Eurofin MWG Operon, Ebersberg Germany) were used as a standard for the quantitative analysis. Standard curves for each target were constructed in a triplex reaction, by using a mix of the same amount of plasmids, in serial

dilutions ranging from 10^1 to 10^7 copies. There was a linear relationship between the threshold cycle values plotted against the log of the copy number over the entire range of dilutions. The copy numbers for individual plasmid preparations was estimated using a Thermo NanoDrop spectrophotometer.

The absolute quantification of total bacterial genome copies in samples allowed for the calculation of the relative amount of red complex species. To prevent samples and polymerase chain reaction contamination, plasmid purification and handling were performed in a separate laboratory with dedicated pipettes.

RESULTS

After the treatment with the zinc-octenidine gel, a remarkable decrease in total bacteria amount and in the individual microbic species was observed in the study group. Specifically *T. forsythia* and *T. denticola* were eradicated whereas the Total Bacteria Loading and *Fusobacterium Nucleatum* showed a percentage reduction of 38% and 55%, respectively (Table I). The zinc-octenedine gel did not show any side effects and was not observed to cause disconfort or to produce adverse reactions in time. No patient reported pain, burning, tingling sensation or numbness. The gel was declared to be tasteless and odorless. Pocket depth at baseline and 15th day was not influenced by the use of the zinc-octenedine gel.

Despite the spread of the data, preventing a correct statistical evaluation, the results obtained clearly demonstrated a reduction of microbial concentration after treatment with the gel.

DISCUSSION

The oral microbiota is constituted by a large number of bacteria species that form a biofilm. The biofilm includes both saprophytes and potentially pathogenic species. It is well understood that most destructive types of periodontal diseases occur due to the presence of pathogenic microorganisms colonizing the subgingival area and the suppression or eradication of these microbes result in improvement in periodontal health. The oral cavity is suitable for invasion of many microorganisms. Mechanical debridement is effective in both spliting the biofilm and reducing the bacterial load. However, the effects of mechanical instrumentation may be improved with the association of a chemical device, providing an additional benefit in controlling the progress of disease.

Local delivery of chemical devices into the pocket achieves a greater local concentration of the drug, proving bactericidal for most periopathogens, and at the same time, exhibiting negligible impact on the microflora residing in other parts of the body (33).

Our study evaluated the efficacy of a hydroxypropyl-cellulose gel containg zinc in association with octenidine as a sustained release drug delivery system in the management of moderate to severe chronic periodontitis.

The results of this investigation demonstrated an overall improvement in bacterial loading at various time intervals. A new dimension was added to this investigation by microbiological examination of bacterial morphotypes using periodontal tests. It was thought appropriate to evaluate the effect of the zinc-octenidine gel on subgingival microbial population, the primary etiological factor for periodontitis. The results demonstrated a marked reduction in total number of organisms in each patient from baseline to 15 days.

A significant reduction was observed in the number of "red complex" bacteria consistent with the antimicrobial activity of the gel. In a previuos *in vitro* study (34) we have demostrated that zinc plus octenedine inhibits the growth of several bacterial species. Gram-positive organisms appear to be more sensitive to zinc-octenedine than Gram-negative bacteria. In particular, the gel is effective against *Streptococcus* groups A, *Staphylococcus aureus*, *Streptococcus* group B. *Escherichia coli, Klebsiella sp., Enterobacter sp. Proteus sp., Pseudomonas aeruginosa, Enterococcus sp*. The zinc-octenedine gel also showed antimicrobial activity against aerobic and anaerobic pathogens and inhibition of S. aureus growth in vitro and in vivo (34).

There is currently a trend in the increased use of antimicrobial agents. The effectiveness of antimicrobial therapies depends on host defence mechanisms and virulence factors. Further research,

with relatively larger sample size and longer follow-up period, will be performed to better validate the efficacy of the zinc- octenedine gel as an effective local drug delivery agent in periodontal therapy.

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