

The use of a new chemical device based on silver and cationic surfactants as a new approach for daily oral hygiene: A preliminary study on a group of periodontal patients

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Abstract

The aim of this study was to evaluate the abatement power of oral microbial loading of a new gel formulation based on the complex silver-2-mercaptobenzoate, chlorhexidine digluconate and didecyldimethylammonium chloride (named ADC) through polymerase chain reaction (PCR). The study sample consists of a group of 20 patients with chronic periodontal disease. Patients were over 25 years of age and did not undergo surgical or non-surgical periodontal treatment in the previous 6 months. The study sample was allotted into two groups of 10 patients each, homogeneous by age and sex. The test group received a bottle containing ADC gel, while the control group received an identical one containing placebo, similar to ADC in consistence, colour, taste and odour. Sub-gingival samples of four sites, one in each quadrant, of greatest probing depth in each patient were used. Microbiological analyses were performed at baseline and at day 15. Paired t test was performed to detect statistical significant reduction in total bacterial loading and oral pathogens in the study groups. The analysis showed a statistically significant reduction in the total bacterial loading evaluated pre- and post-treatment ($P = 0.029$) in the study groups. In the control group, the decrease in total bacterial loading was not significant ($P = 0.279$). Clinically, ADC gel does not have any side effects and discomfort such as pain, burning, tingling sensation or numbness and produces no adverse reactions in time. Our study aimed to evaluate the efficacy of a new chemical formulation with antibacterial properties to use for daily oral hygiene with a preliminary study. Our results showed a statistically significant reduction in total bacterial loading after treatment, but the limitations of our study do not allow us to demonstrate the clinical efficacy of the ADC gel.

Keywords

bacterial load, chronic periodontitis, oral biofilm, red complex

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Introduction

Health of the oral cavity is the result of a balance between bacteria and the immune response. When this balance is broken, pathogenic bacteria (PB) increase, and beneficial bacterial species are suppressed, so periodontal disease may develop.¹⁻⁷

Periodontal treatment consists of mechanical debridement aimed to reduce bacterial loading in the oral cavity.⁸ Periodontal therapy aims to restore a more favourable oral biofilm.⁸ Mechanical debridement reduces the total bacterial loading, but the recolonisation of the oral cavity from PB starts shortly after therapy.⁸ Non-surgical periodontal therapy is widely accepted as a method to keep the natural teeth and preserve oral health, and home oral hygiene is also very important.⁸ The objective of home oral hygiene consists in the elimination of infection and prevention of disease progression. It is now well established that prevention consists in the control of PB, in supportive periodontal therapy, and that the maintenance of oral health is related to proper prevention care programmes. Although preventive protocols are demonstrated to be widely effective, periodontal disease may present relapse caused by poor oral hygiene. The use of topical antimicrobials has proven to be effective for the treatment of periodontal disease in addition to non-surgical periodontal therapy. In particular, nanomaterial has much potential in dentistry due to difficulties with antibiotic resistance.⁹⁻¹² These nanoparticles show synergistic antibacterial and antibiofilm effects in clinical and standard strains.¹¹ Different local drug-delivery systems with chemical devices have been introduced; used at the base of the pocket, they minimise the adverse impact on non-oral body sites.¹³⁻¹⁸

Aim

The aim of this study was to evaluate the efficacy of a new oral gel formulation named ADC (didecyldimethylammonium chloride) in reducing periodontal bacteria using polymerase chain reaction (PCR).

Materials and methods

Gel preparation

The ADC gel is a new formulation based on the complex silver-2-mercaptobenzoate, chlorhexidine

digluconate and didecyldimethylammonium chloride as active ingredients. The silver complex, prepared according to the procedure described in the patent application IT 102018000000576, was mixed with the two organic surfactants in distilled water and the solution was then gelified with hydroxypropyl cellulose and polyvinylpyrrolidone. The gel contains didecyldimethylammonium at 0.5%, chlorhexidine digluconate at 0.1% and Ag⁺ ions at 0.001%. ADC gel is selected for this study for its antibacterial properties as described in the patent application IT 102018000000576.

In vitro microbiological test. A total of 10 specimens were used. The antimicrobial activity of ADC gel was evaluated in vitro against gram-positive and gram-negative bacteria and *Candida albicans*. ADC gel (50 µL) was added in the centre of a Petri dish containing TSA (Tryptone Soya Agar), previously contaminated at the surface with 100 µL of a microbial pool containing *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 15442, *Enterococcus hirae* ATCC 10541 and *Candida albicans* ATCC 1023 in concentration of the order of $1.5 - 5 \times 10^{10}$ cfu/mL (Diagnostic International Distribution S.p.A, Milan, Italy). The minimum inhibitory concentration of a microbial pool (including periodontal pathogen bacteria) in concentration of 10,000,000 cfu/mL was 0.3% w/w. The results of the in vitro test show the remarkable inhibition of the microbial growth observed after 24 h of incubation at 36°C.

Cell viability test. PrestoBlue™ Reagent Protocol (Invitrogen, Carlsbad, CA, USA) was used to evaluate the viability of cells treated with Ag solutions at different concentrations. Cells used for viability test were human gingival fibroblasts.

Cell lines were seeded into 96-well plates at a density of 104 cells per well containing 100 µL of cell culture medium. In all, 1:2 serial dilutions of the stock solution (initial concentration 0.07%) were made with the culture medium.

After 24 h of incubation, cell viability was measured using PrestoBlue reagent protocol (Invitrogen). The percentage of viable cells was determined by comparing the average absorbance in drug-treated wells with the average absorbance in control wells exposed to vehicle alone. The results were presented as the mean \pm standard deviation of three measures.

Experimental design and patient selection

A total of 20 patients with a diagnosis of chronic periodontitis (PD), >25 years, were selected. The exclusion criteria included medically compromised patients, subjects who had been administered antibiotics or antimicrobials in the past 6 months and pregnant and lactating mothers. None of these patients had received any surgical or non-surgical periodontal therapy and demonstrated radiographic evidence of moderate bone loss. The 20 patients were allotted into two groups of 10 patients each, homogeneous by age and sex. The test group received a bottle containing ADC gel, while the control group received an identical one containing placebo, similar to ADC in consistence, colour, taste and odour. All patients were required to perform a proper home oral hygiene for 15 days. During the initial phase, all the subjects were trained about proper home care techniques and monitored during the study period, receiving full mouth scaling and root planning. Sub-gingival samples of four sites, one in each quadrant, of greatest probing depth in each patient were used. The microbiological analyses were performed at baseline and at day 15. The research objectives were explained to the patients, who then signed an informed consent form. This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of 06.09.2013 prot. n. 29579 University Study of L'Aquila.

Microbiological test. LABtest® (LAB SRL®, Ferrara, Italy) was used.¹⁹ It detects *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Fusobacterium nucleatum*, *Campylobacter rectus* and total bacteria loading.

Real-time PCR. Primers and probe oligonucleotides were 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 to find either consensus sequence or less conserved spots. Three real-time PCR runs were performed for each sample. The first reaction quantifies 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 detects and quantifies the three red complex bacteria, that is, *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*, in a multiplex PCR. The third reaction detects and quantifies *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum* and *Campylobacter rectus*. These reactions include six primers each and three probes each that were highly specific for each species. Oligonucleotide concentrations and PCR conditions were optimised to ensure sensitivity, specificity and no inhibition in the case of unbalanced target amounts. Absolute quantification assays were performed using the Applied Biosystems 7500 Sequence Detection System (Thermo Fisher Scientific, Monza, Italy). The amplification profiles were 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 experiments were performed including non-template controls to exclude reagent contamination.

Agents (SI) 125. Plasmids containing synthetic DNA target sequences (Eurofins MWG Operon, Ebersberg, Germany) were standardly used for the quantitative analysis. Standard curves for each target were constructed in a triplex reaction using a mix of the same amount of plasmids in serial dilutions ranging from 10 to 10⁷ 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 (data not shown). The copy numbers for individual plasmid preparations were estimated using the Thermo NanoDrop spectrophotometer (Thermo Fisher Scientific).

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

Statistical analysis

Descriptive statistics were performed using Microsoft Excel 2016 spreadsheets. Paired t test from SPSS program was used to statistically evaluate the change in specific bacteria loading before and after treatment.

Results

The retrieved samples of sub-gingival plaque were investigated to detect the microbial positivity to six different bacterial species and to evaluate the total bacterial loading by quantitative PCR (Table 1). The evaluation showed a statistically significant reduction in total bacterial loading in pre- and post-treatment phases ($P = 0.029$; Table 2) in the experimental group. In the control group, the reduction in total bacterial loading was not significant ($P = 0.279$; Tables 3 and 4).

Discussion

In the oral cavity, bacterial infection induces an inflammatory response with progressive destruction of the periodontal tissues and finally the loss

of teeth. The definition of PD has changed considerably over the years.²⁰ Specific PB of the biofilm formed by plaque causes PD. The PB leak into the periodontal ligament space causing anaerobic infection creates a cascade of events, which ends with the production of inflammatory mediators and bacterial metabolites. PD affects about 50% of adults or more, while the severe form of PD affects around 10% (range: 5%–20%) of adults and the moderate PD around 30%. In the elderly, aged 60–74, the prevalence of PD is estimated at about 70%–90%.²¹

PB associated with periodontal diseases is susceptible to a variety of antiseptics and antibiotics. Non-surgical periodontal therapy, associated with proper domestic oral hygiene, has been documented to preserve the natural dentition by achieving and maintaining a healthy periodontium.²² According to the current state of knowledge, species such as red complex organisms are just a few of those PB that have been shown to play a major role in the pathogenesis of PD.⁶

In this study, a strong reduction in total bacterial loading using ADC for domestic oral hygiene was observed. The reduction in total bacteria can be attributed to the domestic use of the gel and its antibacterial properties.

Since there was no control group, it is difficult to draw final conclusions about the clinical efficiency of ADC, although the reported data show a drop in the total bacterial loading after treatment. It is our knowledge that a randomised controlled trial is mandatory to have final proof about the efficacy of the new molecule to reduce bacterial loading inside the periodontal pocket.

The oral cavity is the interface between the human body and the external world. The external

Table 1. Mean microbial count of specific bacterial species before and after ADC treatment.

	Bacteria	Mean \pm SD
Pair 1	AA1	104.1 \pm 329.2
	AA2	871.6 \pm 2348.3
Pair 2	CR1	123.5 \pm 172.5
	CR2	227.8 \pm 683.3
Pair 3	FN1	2601.9 \pm 2895.5
	FN2	5621.0 \pm 10,188.2
Pair 4	TBL1	505,001.8 \pm 514,769.3
	TBL2	67,015.6 \pm 84,934.2
Pair 5	TD1	83.1 \pm 160.4
	TD2	73.1 \pm 207.2
Pair 6	TF1	111.2 \pm 208.8
	TF2	107.8 \pm 311.2

SD: standard deviation; AA: *Aggregatibacter actinomycetemcomitans*; CR: *Campylobacter rectus*; FN: *Fusobacterium nucleatum*; TBL: total bacteria loading; TD: *Treponema denticola*; TF: *Tannerella forsythia*; 1: before and 2: after treatment.

Table 2. Comparison between total bacterial loading in pre- and post-treatment phases in ADC treated sites.

		Mean \pm SD	95% confidence interval of the difference		Sig.
			Lower	Upper	
Pair 1	AA1-AA2	-767.5 \pm 2413.4	-2494.0	958.9	0.341
Pair 2	CR1-CR2	-104.3 \pm 744.8	637.1	428.5	0.668
Pair 3	FN1-FN2	-3019.1 \pm 11,290.2	-11,095.6	5057.4	0.420
Pair 5	TBL1-TBL2	437,986.2 \pm 534,870.8	55,362.6	820,609.8	0.029
Pair 6	TD1-TD2	11.8 \pm 284.4	-191.7	215.3	0.899
Pair 7	TF1-TF2	3.4 \pm 402.2	-284.3	291.1	0.979

SD: standard deviation; AA: *Aggregatibacter actinomycetemcomitans*; CR: *Campylobacter rectus*; FN: *Fusobacterium nucleatum*; TBL: total bacteria loading; TD: *Treponema denticola*; TF: *Tannerella forsythia*.

layer of the oral epithelium is composed of various epithelia, which constitute barriers guaranteeing the integrity of the organism and protecting it from invasion by PB. It is well known that PD is pathology-associated with PB. Genetic differences in oral plaque, background and general health of patients can control the development of PD.⁶

The association of PD and systemic diseases, such as diabetes, cardiovascular diseases and pre-term birth, reveals the underestimated importance of this disease for global health. A thorough and

early diagnosis of PD allows a more accurate risk calculation for developing systemic pathologies.²³ If a causative relationship is established between PD and these pathologies, therapeutic management of PD will become a part of their prevention.^{24,25}

Conclusion

A wide range of PB has been discovered in various niches in the oral cavity. Their potential roles in regulating the oral environment in both health and disease have also been successfully described in the literature. They have been conserved in evolution and show a relatively higher potential to PD onset, and thus potentially deregulate the biofilm environment and progressing gingival and periodontal diseases. Not just this but also the role of PB in controlling the spread of caries has also been documented. Challenges such as their design, synthesis and function at the molecular level need to be overcome in the near future to open doors towards the design of potentially effective oral microbial antibiotics.

The results of the study provide preliminary data on an overall reduction in bacterial loading with use of ADC ($P = 0.029$). Since there was no control group, it is difficult to draw final conclusions about the clinical efficiency of ADC, although the reported data show a statistically significant drop in total bacterial loading after treatment.

We believe a randomised controlled clinical trial is mandatory to have final proof about the efficacy of the new molecule to reduce specific and total bacterial loading inside the periodontal pocket.

Table 3. Mean microbial count of specific bacterial species of control group treated with placebo.

Comparison between specific bacteria before and after treatment	Bacterial species before and after treatment	Mean \pm SD
Pair 1	AA1	94.2 \pm 29.8
	AA2	416.5 \pm 131.7
Pair 2	CR1	5812.0 \pm 1837.9
	CR2	8271.7 \pm 2615.7
Pair 3	FN1	91,389.2 \pm 28,899.8
	FN2	61,032.8 \pm 19,300.3
Pair 4	PG1	49,860.5 \pm 15,767.3
	PG2	28,850.4 \pm 9123.3
Pair 5	TBL1	1,217,938 \pm 385,145
	TBL2	211,880 \pm 67,002
Pair 6	TD1	11,372.9 \pm 3596.4
	TD2	15,010.1 \pm 4747.6
Pair 7	TF1	6592.1 \pm 2084.6
	TF2	5443.3 \pm 1721.3

SD: standard deviation; AA: *Aggregatibacter actinomycetemcomitans*; CR: *Campylobacter rectus*; FN: *Fusobacterium nucleatum*; PG: *Porphyromonas gingivalis*; TBL: total bacteria loading; TD: *Treponema denticola*; TF: *Tannerella forsythia*; 1: before and 2: after treatment.

Table 4. Comparison between total bacterial loading in pre- and post-placebo phases.

Comparison between specific bacteria before and after treatment	Bacterial species before and after treatment	Mean value and standard deviation	95% confidence interval of the difference		
		Mean \pm SD	Lower	Upper	Sig.
Pair 1	AA1-AA2	-101 \pm 322	-332	128	0.343
Pair 2	CR1-CR2	191 \pm 7361	-5074	5457	0.936
Pair 3	FN1-FN2	3620 \pm 113,755	-77,755	84,996	0.922
Pair 4	PG1-PG2	5211 \pm 55,789	-34,697	45,121	0.774
Pair 5	TBL1-TBL2	386,915 \pm 1,062,470	-373,130	1,146,961	0.279
Pair 6	TD1-TD2	406 \pm 9209	-6181	6994	0.892
Pair 7	TF1-TF2	1454 \pm 4322	-1637	4546	0.315

SD: standard deviation; AA: *Aggregatibacter actinomycetemcomitans*; CR: *Campylobacter rectus*; FN: *Fusobacterium nucleatum*; PG: *Porphyromonas gingivalis*; TBL: total bacteria loading; TD: *Treponema denticola*; TF: *Tannerella forsythia*.

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