

A hydrosilver gel for plaque control in adults affected by chronic periodontitis: Effects on the 'red complex' bacterial load. A prospective longitudinal pilot study using polymerase chain reaction analysis

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Abstract

In subjects affected by chronic periodontitis, the chemical control of plaque is a strategy aiming primarily at controlling infection and bacterial loading. The aim is to evaluate the bacterial loading of the so-called 'red complex' associated with a short-term use of a hydrosilver gel (HSG) by using an *in vivo* model in adult subjects affected by chronic periodontitis. This prospective short-term clinical trial involved 10 adult volunteers using a 15-day *in vivo* model. After receiving professional prophylaxis at baseline (t0), each volunteer performed daily applications of HSG at home. After 15 days (t1) from the first application, subgingival plaque samples were collected, and the bacterial loading of species belonging to the red complex was evaluated using polymerase chain reaction (PCR) analyses. The bacterial loading of the red complex showed no statistically significant difference between t0 and t1, although it tended to decrease. HSG can be used at home as an adjunct to domestic oral care because it seems a promising tool, but further studies are needed to involve a larger sample and a longer follow-up.

Keywords

bacterial loading, chronic periodontitis, domestic oral hygiene, hydrosilver gel, red complex

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Background

Periodontitis is a family of diseases affecting dental supporting tissues, caused by infections sustained by periodontal pathogens and also related to an immune response,^{1,2} which leads to soft and hard tissue destruction, dental mobility and, finally, to the loss of dental elements. It can also be correlated to systemic diseases such as metabolic syndrome,³ and it can be considered as a condition with increased serum levels of products derived from oxidative damage, promoting a generalized proinflammatory state.²

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In chronic periodontitis, the oral polymicrobial communities might evolve from a phenomenon called ‘dysbiosis’, that is, a change in the proportion of saprophytes and pathogens. In ‘dysbiosis’, the exact identification of the pathogenic species and their relative proportions has not been completely clarified yet.⁴

Probably, this situation could be due to the particularity of the intra-oral environment, in which various materials can be present, both organic and/or not, as the dental rehabilitations (prosthetic materials, metals, ceramics, composites, etc.).

Some of these materials remain in the mouth for a medium–short time (e.g. the orthodontic appliances), while others remain ideally ‘for life’ (implant-prosthetic rehabilitations). For some of them, it was observed that their presence influences the composition of the bacterial flora.^{5,6}

The main pathogens of periodontitis have been identified in bacteria belonging to the so-called ‘red complex’, that is, *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*, which is considered as the main cause of changes in polymicrobial communities.

Bacteria of the ‘orange complex’ are *Fusobacterium nucleatum*, *Campylobacter rectus*, *Aggregatibacter actinomycetemcomitans*, *Atopobium rimae*, *Eubacterium saphenum*, *Porphyromonas endodontalis* and *Treponema lecithinolyticum*. These are the main components of microbiological shift.

In chronic periodontitis, one of the most important clinical strategies is the control of the domestic oral hygiene (DOH), in order to preserve the normal oral microbiota.⁷ The DOH is often accompanied by the use of chemical substances, such as mouthrinses⁸ and/or gel,⁹ to control the bacterial growth.

The objective of DOH is the elimination of infection and prevention of disease progression. It is now well established that prevention in supportive periodontal therapy consists in the control of bacterial plaque and that the maintenance of oral health is related to proper prevention care programmes. Although the DOH is demonstrated to be widely effective, periodontal disease (PD) may present relapse caused by poor oral hygiene.

For example, however, the level of hygiene was also related to the survival of long-term implants in patients treated with chronic periodontitis,⁷ mostly in subjects with systemic diseases.¹⁰

Thus, the chemical control of plaque is considered a strategy that can be added to manual manoeuvres and can be crucial for achieving and/or improving plaque control during DOH. The clinicians are therefore increasingly looking for new molecules that – used in the form of gels or mouthwashes – can support oral hygiene manoeuvres, especially in adult patients with chronic periodontitis. Among these, a promising gel seems to be the hydrosilver gel (HSG).

Specific objective

The specific objective of this study is to evaluate the bacterial loading of the so-called red complex associated with a short-term use of a HSG using an *in vivo* model in adult subjects affected by chronic periodontitis.

HSG composition

HSG contains a 0.1% w/w of silver ions, Ag⁺, a 0.14% of thiosalicylic acid, sodium salt and a 1.6% of sodium hyaluronate swollen in water. The silver ions are bound to the salicylate anion to form a negatively charged complex, which is water soluble and compatible with the presence of sodium hyaluronate.

Materials and methods

Trial design

This prospective short-term clinical trial involved 10 adult volunteers, using a 15-day *in vivo* model. No changes in methods after the trial beginning (such as eligibility criteria) were performed. 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 (Italy).

Participants

In all, 10 patients were randomly selected with a diagnosis of chronic periodontitis. All patients were volunteers participating in the study. All of the candidates were screened for suitability by the research team. The inclusion criteria were age >25 years, probing depth of 3 mm or more and dentate with >20 natural teeth. The exclusion criteria were medically compromised patients, patients who have been administered

Table 1. Hydrosilver gel composition.

Hydrosilver gel	Water 0.1% w/w of silver ions, Ag ⁺ 0.14% of thiosalicylic acid, sodium salt 1.6% of sodium hyaluronate swollen in water
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antibiotics or antimicrobials in the past 6 months, smokers and pregnant lactating women. All eligible volunteers were given oral and written information about the product and the purpose of the study and were asked to sign an informed consent form.

Interventions

At t₀, after baseline examinations consisting of an oral soft and hard tissue examination, the subjects received complete dental prophylaxis including scaling and polishing to remove all the plaque and extrinsic tooth stains. They were given the proper DOH instructions. The subjects then received the bottles of HSG (Table 1). All the participants were instructed not to use any other oral hygiene measures during the experimental period and to perform the correct manoeuvres and to apply the HSG after DOH once daily in the evening. The application of the HSG was performed at home without supervision. After 15 days (t₁), all the volunteers were examined, and microbiological samples were collected from periodontal pockets.

Clinical methods

All participants were evaluated clinically. The same examiner assessed the clinical and periodontal parameters. Probing depth measured to the nearest millimetre from the gingival margin to the bottom of the pocket was noted using calibrated William's periodontal probe for all measurements. A total of 20 sites were selected from the 10 patients. For each patient, two sites were deep periodontal pockets, hence greater index of PD.

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 day), 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 and the distance between the base of the pocket and gingival margin were 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 subgingival 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: *P. gingivalis*, *T. forsythia* and *T. denticola*.

Microbiological test

The LAB[®]-Test (LAB s.r.l.[®], Ferrara, Italy) was employed to detect the presence and the level of the pathogens. This is a rapid and sensitive test to detect and quantify the three bacterial species more involved in periodontitis that constitute the 'red complex', that is, *P. gingivalis*, *T. forsythia* and *T. denticola*.

Both *P. gingivalis* and *T. denticola* are considered the first pathogens involved in the clinical destruction of periodontal tissues and appear with the first clinical signs of periodontal destruction. They appear 'linked' in the biofilm due to their ability to produce a number of outer membrane-associated proteinases, previously demonstrated *in vitro*.

Moreover, together with *T. forsythia*, they show a higher prevalence in affected subjects rather than in healthy ones, suggesting that these bacteria may be associated with the local development of periodontitis. They can be revealed by real-time polymerase chain reaction (PCR) analysis using bacterial species-specific primers and probes.

In addition, the following bacteria of the 'orange complex' were monitored: *F. nucleatum*, *C. rectus*, *A. actinomycetemcomitans* and *T. lecithinolyticum* as the main component of microbiological shift.

Real-time PCR

Primers and oligonucleotide probes were designed on the base of a 16S ribosomal RNA (rRNA) gene sequence of the Human Oral Microbiome Database (HOMD 16S rRNA RefSeq Version 10.1), counting 845 entries. All the sequences were aligned in order

Table 2. Bacterial loading of the species belonging to the 'red complex' at t0 and t1 with comparisons.

		Mean (\pm SD)	Difference t0-t1 Mean (\pm SD)	P value
<i>Porphyromonas gingivalis</i>	t0	48.3 (\pm 152.7)	-48.3 (\pm 152.7)	n.s.
	t1	0		
<i>Treponema denticola</i>	t0	133.2 (\pm 268.1)	-60.1 (\pm 152.7)	n.s.
	t1	73.1 (\pm 118.9)		
<i>Tannerella forsythia</i>	t0	207.9 (\pm 337.6)	104.6 (\pm 152.7)	n.s.
	t1	312.5 (\pm 489.9)		

n.s.: not significant.

to find either consensus sequence or less conservative spots. Two real-time PCR runs were performed for each sample. 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 rRNA gene. The second reaction detected and quantified the three 'red complex' bacteria, namely, *P. gingivalis*, *T. forsythia* and *T. denticola*, in a multiplex PCR. A total of six primers and three probes were employed, which are highly specific for each species. 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 using the Applied Biosystems 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The amplification profile was initiated by a 10-min incubation period at 95°C to activate polymerase, followed by a two-step amplification of 15s at 95°C and 60s at 57°C for 40 cycles. All these experiments were performed including non-template controls to exclude reagent contamination.

Plasmids containing synthetic DNA target sequences (Eurofin MWG Operon, Ebersberg Germany) were used as 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 10¹ to 10⁷ copies.

The copy numbers for individual plasmid preparations were estimated using the Thermo Scientific NanoDrop spectrophotometer. The quantification of the total bacterial genome copies in the samples allowed the calculation of the relative amount of the red complex. To prevent samples and PCR contamination, plasmid purification and handling were performed in a separate laboratory with dedicated pipettes.

Data analysis

The data were analysed for normality of distribution with the Shapiro-Wilk test. Because the resultant data were not normally distributed, non-parametric analysis was performed (the Wilcoxon signed-rank test) to detect any significant difference between the bacterial loading at t0 and t1, for each of the three species. A 5% significance level was adopted, and the data analysed using the Stata/IC 12.1 statistical package.

Results

Microbiological results

The number of 'red complex' organisms, such as *P. gingivalis*, *T. forsythia* and *T. denticola*, was analysed after 15 days from the beginning of the study (t0 vs t1 comparisons; Table 2). Data about the bacterial loading of *F. nucleatum*, *C. rectus*, *A. actinomycetemcomitans* and *T. lecithinolyticum* are reported in Table 3. Total bacteria loading resulted not significantly reduced.

Discussion

According to the current state of knowledge, species such as the 'red complex' organisms have shown to play a major role in the pathogenesis of periodontitis.¹¹ The gold standard for the management of chronic periodontitis is the correct DOH.

Adjunctive molecules to DOH, like local drug delivery, have been used in conjunction to this in order to improve the therapeutic results, and their application has gained increased attention in recent years.^{9,12-14}

This study reveals that the HSG is associated with a decreased tendency of bacterial loading. However, the microbiological analyses failed to

Table 3. Bacterial loading of the species belonging to the 'orange complex' at t0 and t1 with comparisons.

		Mean (\pm SD)	Difference t0-t1 Mean (\pm SD)	P value
<i>Fusobacterium nucleatum</i>	t0	8051.6 (\pm 152.7)	8168.7 (\pm 38,125.9)	n.s.
	t1	16,220.3 (\pm 34,695.5)		
<i>Campylobacter rectus</i>	t0	870 (\pm 1737.5)	-693.3 (\pm 1663.8)	n.s.
	t1	176.7 (\pm 316.8)		
<i>Aggregatibacter actinomycetemcomitans</i>	t0	726.4 (\pm 981.2)	-540.5 (\pm 1268.2)	n.s.
	t1	185.9 (\pm 587.9)		
<i>Treponema lecithinolyticum</i>	t0	2,504,973 (\pm 4,197,942)	-1,792,607 (\pm 3,136,375)	n.s.
	t1	712,366 (\pm 1,451,068)		

detect a statistically significant reduction of the bacterial loading of the red complex species. LAB-test can be considered a useful tool to monitor the effects of mechanical and/or chemical therapy on the subgingival microflora, as PCR assay has been shown to be highly sensitive and specific for the periodontal pathogens.¹⁵

The effect of the HSG is probably associated with its ability as biocide. In this study, we explored the antimicrobial activity of aqueous solutions containing a thermally and photochemically stable anionic silver complex, where the silver ion is mixed with salicylate anion. Silver complex antimicrobial activity occurs damaging cells by transporting the molecules into the membrane through pores, ion channels, ion pumps or specific carriers. Previous studies have testified the existence of a synergic antimicrobial action of silver complex.¹⁵

In the oral cavity, the lack of DOH 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.

The association of PD and systemic diseases, such as diabetes and cardiovascular diseases, reveals the underestimated importance of this disease for global health. Another possible application of HSG would be in the treatment of peri-implantitis. In fact, dental implants had a great success in the last decades for replacing missing teeth in partially or totally edentulous patients. Even if the main factor for implant dentistry success is the quality of bone of receiving sites, the bacteria of PD also cause peri-implantitis.¹⁰

This study is limited by the small sample size that involved only volunteers and the short follow-up. The hydrogel seems to be a promising tool as an adjunct chemical device of DOH in subjects

affected by chronic periodontitis. As the use of effective local drug delivery agents is advocated by clinicians to improve the efficacy of DOH for the infection control, further studies on the efficacy of HSG are requested.

Declaration of conflicting interests

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