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Letter to the Editor

Reuterinos® as adjuvant for peri-implant treatment: A pilot study

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Abstract

The objective of this study was to evaluate the effects of lozenges-containing *Lactobacillus reuteri* as an adjuvant treatment of peri-implant mucositis and to detect the level of *L. reuteri* colonization in the peri-implant tissues of treated patients. A total of 10 patients were selected. Subjects with at least one implant affected by peri-implant mucositis, with gingival index (GI) of ⩾2 in each quadrant, evaluated at the buccal aspect of all teeth. Patients included in the study were partially edentulous and had implants with mucositis or peri-implantitis. Implants with radiographic bone loss of ⩾5 mm and/or ⩾50% of the implant length were excluded, and only one implant per patient was included. Each patient received *L. reuteri*–containing lozenges. Microbiological sampling was performed at baseline and on day 28 and analysed by polymerase chain reaction (PCR). Our results indicate that the use of the probiotic did not influence the peri-implant microbiota in a statistically significant way, although there was a reduction in the number of periodontal and peri-implant species. The lack of statistically significant microbiological changes could be explained either by the small sample population or by the short evaluation period. Therefore, the poor colonization of *L. reuteri* in the peri-implant pockets can be explained by the different anatomical and histological characteristics of the interface of the dental–gingival unit with respect to the periodontal sulcus. The administration of a daily lozenge of *L. reuteri* for 4 weeks had a limited effect on the microbiological analysis. Probiotics provide an alternative therapeutic approach to consider in the prevention and treatment of peri-implant diseases, but further long-term prospective studies with standardized variables are needed.

Keywords

gingivitis, *L. reuteri*, mucositis, oral microbiota, probiotic

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Introduction

Mucositis is not a disease that needs to be treated, but rather a disease that should be prevented. In order to prevent mucositis, everyone should know what causes this disease. The occurrence rate of mucositis is high,[1] even with patients who pay attention to oral hygiene.

Peri-implantitis is an infection of both bone and soft peri-implant tissues. It is a pathology that can occur at a distance of 3–9 years and which leads to the progressive reabsorption of the bone surrounding the implant, further leading to total loss of the implant.[1] The implant loss is related to major bone

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defects; it is difficult to position a new implant with a new tooth. Peri-implantitis is a disease very similar to periodontitis,[1] always mediated by bacteria, and therefore must be treated in a similar way, with a series of protocols that lead to the elimination of infection and bacteria within the implant.[1]

The main causes of peri-implantitis are pathogenic bacteria;[1] hence, it is very important to remove or prevent these bacteria that tend to accumulate on the implant surface, especially if it is rough. Another important factor is the individual predisposition of the patient: there are patients who often fall sick, especially smokers, and patients who, instead, have a lower tendency.[1] Thus, the individual response component of the host is important too.[1] The implant surface is another possible cause of peri-implantitis: a smoother surface is less susceptible to infection of the peri-implant tissues and therefore leads to peri-implantitis.[1]

Prevention of peri-implantitis is mandatory because once the disease has manifested, it is very difficult to eradicate and control. To prevent peri-implant mucositis, it is important to use implants that do not have excessively rough surfaces that therefore could favour the accumulation of bacteria on the implant surface; in particular, it is important that the patient undergoing implant therapy is then followed with hygiene protocols every 3–6 months by the professional or even by dental hygienists in order to avoid excessive accumulation of plaque at the level of the implant surface and peri-implant tissues.

There are two phases of treatment for peri-implant mucositis: initially, through the action of a dental hygienist, it is necessary to remove the greatest amount of bacteria from the tissues and subgingival areas of the implant surface and thus maintain this health condition for as long as possible (even forever). The patient must perform a scrupulous oral hygiene, and if this is not enough, it is necessary to perform surgical therapies, detaching the peri-implant tissues and soft tissues, smoothing the implant surface and remove all the bacteria. Then, it is necessary to close the gingival tissues and eventually, in some cases, regenerate the bone or part of the bone that has been lost.

Probiotics for peri-implantitis prevention

Probiotics are defined as living and viable microorganisms which, when administered in adequate quantities, confer benefits to the organism. They can interact positively with the intestinal immune system and help prevent gastrointestinal disorders.

When our intestinal flora loses its balance, the bad bacteria take the upper hand over the good ones (dysbiosis): this is the moment when it becomes very important to introduce probiotics to restore the correct balance. To achieve this goal, it is necessary to take products based on probiotics that are able to survive the acidity of the gastric environment, reach the intestine and fight the harmful germs, restoring the balance of the intestinal flora, by adhering to the intestinal mucosa and carrying out beneficial actions. The use of probiotics has not been proposed for mucositis treatment and prevention.

To the best of our knowledge, probiotics efficacy on the oral microflora preventing the colonization of periodontal pathogens, and thus preventing the microbiological shifts associated with mucositis, has not been investigated.

Probiotic tablets containing *Lactobacillus reuteri* have been formulated with the aim of preventing and treating gingivitis, although to the investigator's knowledge, there are no controlled studies evaluating its efficacy.[2–4]

Therefore, the purpose of this investigation is to study the clinical and microbiological effects of *L. reuteri* and to evaluate the patterns of colonization in peri-implant pockets.

Materials and methods

Subjects

A total of 10 healthy volunteers were recruited among patients of a private practice from February to October 2017, provided they fulfilled the following criteria:

- Subjects with at least one implant affected by peri-implant mucositis, with gingival index (GI) of $\geqslant 2$ in each quadrant, evaluated at the buccal aspect of all teeth. Patients included in the study were partially edentulous and had implants with mucositis or peri-implantitis. Implants with radiographic bone loss of $\geqslant 5$ mm and/or $\geqslant 50\%$ of the implant length were excluded, and only one implant per patient was included.
- Subjects were excluded if they had used any systemic antibiotics in the previous 3 months, probiotic preparations or oral antiseptics in the previous month or if they had any

systemic disease or condition that could interfere with the study results (e.g. diabetes and immunological disorders, pregnancy, ongoing drug therapy that could affect the signs of mucositis).

Study design

All selected subjects signed an informed consent to participate in the study. 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. After non-surgical mechanical therapy, subjects were randomly assigned to take either one probiotic lozenge or one placebo lozenge every day for 4 weeks. Participants were asked not to change their oral hygiene habits and to refrain from taking other probiotic products throughout the duration of the study. A total of 10 patients were selected. Clinical measurements were taken in the whole mouth (GI) and at the implant site (probing pocket depth) at baseline and after 4 weeks. Microbiological examination was performed at the same study time points that clinical measurements were made. Each selected site will be subjected to microbial analysis. For the collection of subgingival samples, the site was 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. They were instructed on the use of the tablet medications (Reuterinos®; Noos s.r.l., Rome, Italy) and were scheduled for new evaluations, after 4 weeks, with additional clinical and microbiological examinations. After 4 weeks, they were scheduled for a baseline examination. At this visit, the clinical and microbiological examinations were carried out.

Treatments

Subjects were randomly assigned following a computer-generated randomization list. Each subject identified by a unique study number was instructed to chew one tablet per day (Reuterinos; Noos s.r.l.), during 28 days.

Clinical examination

Clinical variables were evaluated at baseline and 4 weeks. The variable included the GI,[5] as normally assessed in studies evaluating oral hygiene products. The same examiner evaluated this index by selecting randomly in each patient two quadrants: either upper right and lower left or upper left and lower right quadrants (half of the mouth scoring).[5]

Microbiological analysis

The microorganisms processed were the three bacterial species, which were involved in most of the periodontitis cases, that constitute the red complex group: *Porphyromonas gingivalis, Tannerella forsythia* and *Treponema denticola*, as described in previous studies.[6–9]

Both *P. gingivalis* and *T. denticola* occur concomitantly with the clinical signs of periodontal destruction.[1] They appear closely 'linked' topologically in the developing biofilm, with an in vitro ability to produce a number of outer membrane–associated proteinases, and are considered the first pathogens involved in the clinical destruction of periodontal tissues. Moreover, *P. gingivalis* and *T. denticola* and *T. forsythia* show a higher prevalence in disease than in health suggesting that these bacteria are associated with the local development of periodontitis.[1]

Real-time polymerase chain reaction. Primers and oligonucleotide probes will be designed based on 16S ribosomal RNA (rRNA) gene sequences of the Human Oral Microbiome Database (HOMD 16S rRNA RefSeq Version 10.1) counting 845 entries. All the sequences will be aligned in order to find either consensus sequence or less conservative spots. Two real-time polymerase chain reaction (PCR) runs will be performed for each sample. The first reaction will quantify the total amount of bacteria using two degenerate primers and a single probe matching a highly conservative sequence of the 16S rRNA gene. The second reaction will detect and quantify the three red complex bacteria, that is, *P. gingivalis, T. forsythia* and *T. denticola*, in a multiplex PCR. This reaction will include a total of six primers and three probes that are highly specific for each species. Oligonucleotide concentrations and PCR conditions will be optimized to ensure sensitivity, specificity and no inhibitions in case of unbalanced target amounts. Absolute quantification assays will be performed using the Applied Biosystems 7500 Sequence Detection System. The amplification profile will be 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 these experiments will be performed including nontemplate controls to exclude contamination of reagents.

Plasmids containing synthetic DNA target sequences (Eurofin MWG Operon, Ebersberg, Germany) will be used as standard for the quantitative analysis. Standard curves for each target will be constructed in a triplex reaction, using a mix of the same amount of plasmids, in serial dilutions ranging from 101 to 107 copies. There is 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 will be estimated using the Thermo NanoDrop spectrophotometer.

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

Statistical analysis

Descriptive statistics were performed using Microsoft Excel spreadsheets. The Freeman–Halton extension of Fisher's exact test was used to compute the (two-tailed) probability of obtaining a distribution of values in a 2×3 contingency table, given the number of observations in each cell. Odds ratio calculation was performed online at the OpenEpi website (www.openepi.com).

Results

After treatment, there was a reduction in specific and total bacterial loading, although no statistical significant difference was detected (Tables 1 and 2). After 4 weeks, GI values were better to baseline as well as gingival inflammation.

Table 1. Mean amounts of specific bacterial species before and after Reuterinos® treatment.

	Mean	N	SD	SEM
Pair 1 AA1	0.0000[a]	10	0.0000	0.0000
Pair 1 AA2	0.0000[a]	10	0.0000	0.0000
Pair 2 CBT1	160,389.7	10	222,067.0	70,223.76
Pair 2 CBT2	12,022.00	10	10,683.94	3378.5570
Pair 3 CR1	1491.5000	10	3998.3617	1264.3930
Pair 3 CR2	7.2000	10	22.7684	7.2000
Pair 4 FN1	26,432.80	10	72,830.50	23,031.03
Pair 4 FN2	414.8000	10	500.8936	158.3965
Pair 5 LR1	13.9000	10	15.3511	4.8544
Pair 5 LR2	16.1000	10	13.2535	4.1911
Pair 6 PG1	8.6000	10	27.1956	8.6000
Pair 6 PG2	0.0000[a]	10	0.0000	0.0000
Pair 7 TD1	0.0000[a]	10	0.0000	0.0000
Pair 7 TD2	0.0000[a]	10	0.0000	0.0000
Pair 8 TF1	106.6000	10	337.0988	106.6000
Pair 8 TF2	8.0000	10	25.2982	8.0000

SD: standard deviation; SEM: standard error of the mean.
[a]The correlation and t cannot be computed because the standard error of the difference is 0.

Discussion

Lactobacillus reuteri is a species of bacterium belonging to the Lactobacillaceae family that naturally colonizes the gastrointestinal tract of humans and animals. Some clinical studies have shown that adequate administration of *L. reuteri* can bring benefits to human health.[10,11] For this reason, *L. reuteri* is currently considered a probiotic organism. Some strains of *L. reuteri* (mainly ATCC55730 and DSM17938) are currently used as therapeutic agents against various intestinal disorders.

Lactobacillus reuteri, belonging to the genus *Lactobacillus*, is a gram-positive bacterium, which, due to its unique metabolic properties, belongs to the group of lactic bacteria (also called 'lactic ferments') that colonize both men and animals. In humans, it has been isolated in the gastrointestinal tract and in samples of faecal and vaginal material. It is also present in breast milk, together with other lactic bacteria of the genera *Lactobacillus* and *Bifidobacterium*. Based on the study by Sinkiewicz,[10] who considered more than 200 women from seven different countries in the world, *L. reuteri* was isolated in human milk in both urban and rural areas, with colonization rates of up to 50%.

One of the most studied Lactobacilli and having great effectiveness today is *L. reuteri*, described for the first time by Gerhard Reuter in 1980: commonly already present in the intestinal mucosa since the first hours of life, *L. reuteri* is part of that important immunity that is transmitted from the mother to the baby also through the mother's milk.[11]

The experimental design of this clinical trial aimed to study the clinical and microbiological impact of the use of probiotic tablets containing *L.*

Table 2. Output of paired samples t test.

	Paired differences					t	df	Sig. (two-tailed)
	Mean	SD	SEM	95% confidence interval of the difference				
				Lower	Upper			
Pair 2 CBT1-CBT2	148,367.7	217,033.17	68,632.09	−6888.87	303,624.33	2.162	9	0.059
Pair 3 CR1-CR2	1484.3000	4000.6698	1265.1229	−1377.61	4346.2068	1.173	9	0.271
Pair 4 FN1-FN2	26,018.00	72,759.78	23,008.66	−26,031.2	78,067.21	1.131	9	0.287
Pair 5 LR1-LR2	−2.2000	2576.73	8.1483	−20.6328	16.2328	−0.270	9	0.793
Pair 6 PG1-PG2	8.6000	2719.56	8.6000	−10.8546	28.0546	1.000	9	0.343
Pair 8 TF1-TF2	98.6000	340.8382	107.7825	−145.2210	342.4210	0.915	9	0.384

SD: standard deviation; SEM: standard error of the mean.

reuteri and further to assess the patterns of *L. reuteri* colonization in peri-implant pockets. Similar to another study,[12] our results indicate that the use of the probiotic did not influence in a statistically significant way the peri-implant microbiota, although there was a reduction in the number of periodontal and peri-implant species. The lack of statistically significant microbiological changes could be explained either by the small sample population or by the short evaluation period. Therefore, the poor colonization of *L. reuteri* in the peri-implant pockets can be explained by the different anatomical and histological characteristics of the interface of the dental–gingival unit with respect to the periodontal sulcus.

The administration of a daily lozenge of *L. reuteri* for 4 weeks had a limited effect on the microbiological analysis. Probiotics provide an alternative therapeutic approach to consider in the prevention and treatment of peri-implant diseases, but further long-term prospective studies with standardized variables are needed.

Declaration of conflicting interests

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