The arginine-deiminase enzymatic system on gingivitis: preliminary pediatric study

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Summary
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Aim. The lactic bacteria are Gram-positive microorganisms with coccus or stick shape, which share a number of physiological and biochemical properties. Several experimental evidences suggest the possibility of using lactic acid bacteria as a potential preventive or therapeutic approaches, alternative or complementary protocols prevention or treatment currently followed in several pathological conditions.

Introduction
The lactic bacteria are Gram-positive microorganisms with coccus or stick shape, which share a number of physiological and biochemical properties. They include some species of lactobacilli, lactococci, streptococci, pediococci. Several experimental evidences suggest the possibility of using lactic acid bacteria as a potential preventive or therapeutic approaches, alternative or complementary protocols prevention or treatment currently followed in several pathological conditions (Offenbacher, 1993; Mombelli, 1998). Specific components of lactobacilli seem to have an intense effect on the modulation of immune response, on the activation of the reticuloendothelial system and on the regulating production of cytokines and inflammatory response (Malin, 1996; Naidu, 1999). In this way it is very important to the study conducted by Ulisse (Ulisse, 2001) in which was found an inhibitory action of Lactobacillus brevis against iNOS, in rat’s peritoneal macrophages stimulated in vitro with IFN-γ and lipopolysaccharide (LPS). In the light of these results, in this work, we have proposed to assess the anti-inflammatory effects of Lactobacillus brevis in case of marginal gingivitis.

Materiale and methods. In our study were examined 21 subjects, 16 males and 5 females, aged between 5 and 12 years, with marginal gingivitis problems who have been given chewing gum containing the principle to test in vivo L. brevis anti-inflammatory effect choosing as experimental model the gingivitis.

Results. At the time T1, after treatment, 18 patients no longer showed inflammation; 2 of them had a slight inflammation and only 1 patient still showed a moderate inflammation.

Conclusions. From our research, as confirmed by clinical and laboratory investigation, results an effective anti-inflammatory action of arginine-deiminase system that some bacteria possessing.

Key words: gingivitis, lactobacillus brevis, arginine-deiminase.
To analyze the in vivo effects of the tablets containing *L. brevis* on clinical signs in patients with primitive and applicant marginal gingivitis.

To analyze the in vivo effects of the tablets containing *L. brevis* on the salivary IgA levels and on the nitric oxide synthase (NOS) activity (Fig. 1).

**Materials and Methods**

In our study were examined 21 subjects, 16 males (76%) and 5 females (24%), aged between 5 and 12 years, with marginal gingivitis problems, 16 volunteers as controls, 10 males (62.5%) and 6 females (37.5%), examined at the U.O.C. of Paediatric Dentistry of “Sapienza”, University of Rome. The controls were used only for the evaluation of laboratory data, and not in clinical investigations. To patients were administered gums containing the active ingredient (Fig. 2) for a period of 60 days. The procedures were carried out in accordance with the Helsinki Declaration of 1975, and the 1983 revision of the same. For the study a medical bill in different sections was drawn up.

**Section 1**

- **Personal data.**
- Accurate family history, physiological and pathological remote and next.
- Possible presence of underlying conditions; excluding subjects with systemic diseases or subjects under drug prolonged treatments.
- Personal behavior including the possible presence of orthodontic appliances.

They were selected subjects suffering from marginal gingivitis (Fig. 3) (T0) who have been given chewing gums containing the principle to test in three per day. The gums, taken at regular intervals, were chewed by patient for a period longer than 20 minutes. The checks were made at the time T1 (30 days) and T2 (60 days). During each control has been made a levy saliva. The saliva samples of patients and controls, collected in tubes before and after treatment and immediately frozen at -20°C until use, were analyzed for the activity of NOS (this was determined as levels of nitrites/nitrates in the hamlet of salivary fluid), and the levels of IgA (Giuc, 2007; Grbic, 1995; Haffajee, 1983; Heskens, 1996; Riccia, 2007).

**Section 2**

The clinical evaluation was performed referring on 6 dental elements (upper arch: earlier sector 1.1 or 1.2, medium-rear sector 1.6 and 2.6; lower arch: earlier sector 3.1 or 4.1, medium-rear sector 3.6 and 4.6), with symptoms (thermal sensitivity and bleeding) and objectivity (plaque, scale, gingival inflammation) recorded at the time T0, T1 and T2 above reported with a gradual scale (Tables 1 and 2).

**Section 3**

Registration at T1 and T2 of possible side effects resulting from the administration of the principle tested.

**Section 4**

Final evaluation including clinical and laboratory data. With regard to clinical aspects on the basis of results obtained has given judgment:

- healing;
- improvement;
- failure;
- relapse;
- do not assessable.

**Results**

The clinical signs and symptoms (gingival inflammation, plaque, scale, sensitivity to temperature and bleeding after
pressure), given before and after treatment with the gums of L. brevis, are shown in Table 3 and Table 4. At the time T0, before treatment, gingival inflammation was evaluated moderate/diffused in 20 patients and slight in 1 patient (Table 3 and Figure 4). At the time T1, after treatment, 18 patients no longer showed inflammation; 2 of them had a slight inflammation and only 1 patient still showed a moderate inflammation. The tolerance to the treatment was considered very good in all patients. Overall dentist and patients opinion coincided and the results confirmed regression in 18 patients and improved in the remaining 3 of them. The effects of treatment on the levels of plaque and scale are shown in Table

Table 1 - Symptoms with gradual scale shown in Section 2.

<table>
<thead>
<tr>
<th></th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>Absent</td>
<td>Slight</td>
<td>Moderate</td>
</tr>
<tr>
<td>P</td>
<td>Absent</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
</tbody>
</table>

Table 2 - Signs objectives shown in Section 2.

<table>
<thead>
<tr>
<th></th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>Absent</td>
<td>Slight</td>
<td>Moderate</td>
</tr>
<tr>
<td>P</td>
<td>Absent</td>
<td>Moderate</td>
<td>Diffused</td>
</tr>
</tbody>
</table>

Table 3 - Effect of gums with L. brevis on gingival inflammation, plaque and scale.

<table>
<thead>
<tr>
<th></th>
<th>Scale</th>
<th>Gingival inflammation</th>
<th>Plaque</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>A, B, H, M,</td>
<td>A, C, D, F, G, I, L,</td>
<td>T, D, F</td>
</tr>
<tr>
<td>Slight</td>
<td>P, Q</td>
<td>O, R, T, U, V, Z</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>C, D, E, F,</td>
<td>C, D, E, F, G, I, L,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>O, R, T, U,</td>
<td>A, C, D, F, G, I, L,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V, Z</td>
<td>O, R, T, U, V, Z</td>
<td></td>
</tr>
<tr>
<td>Diffused</td>
<td>A, B, V, E,</td>
<td>A, B, V, E, H, M, O,</td>
<td></td>
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<td></td>
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3, which notes the improvement in the levels of plaque in almost all patients. In Table 4 (Figure 5) are shown the beneficial effects of treatment on sensitivity to temperature and on bleeding after pressure.

Evaluation of secretory IgA

They were analyzed levels of secretory IgA in saliva fluid fraction of control subjects and in patients with gingivitis before (T0) and after (T1) treatment with the gums. In Tables 5 and 6 and in Figure 6 are showed the data about antibody. A significant reduction in IgA levels was founded in saliva of the patients compared to the control samples, while treatment with tablets not producing a significant change.

Evaluation of nitrite/nitrate levels

Levels of nitrites/nitrates are often indicative of NOS activity. In our study we proposed to detect the activity of this enzyme. The results of determining the levels of nitrites and nitrates in the saliva of healthy subjects and subjects with gingivitis before (T0) and after (T1) treatment with the gums are reported in Tables 5 and 6 and in Figure 5. As it is possible to see levels of nitrites/nitrates in patients with gum disease were significantly higher (P<0.01) compared to those of controls. It seems clear that in all patients, treatment with the gums with *L. brevis* was associated with a significant reduction in the levels of nitrites and nitrates (P <0.01).

Conclusions

*Lactobacillus brevis* is able, through the arginine-deiminase activity, subtract the substrate (arginine) to nitric oxide synthase and to inhibit in vitro the generation of nitric oxide from rat’s peritoneal macrophages stimulated with LPS and IFN-γ. These data lead us to study in vivo the *L. brevis* anti-inflammatory effect choosing as experimental model the gingivitis. The choice was dictated by some observations and needs. *L. brevis*, like other lactic bacteria, is part of the normal flora of the oral cavity, is present in dental plaque and in literature is not reported as pathogen for periodontium; the method of collection of samples was not painful for patients and the inflammatory state and evolution of the disease were evaluated using an objective examination and with the use of a simple equipment. Overall, our results allow us to hypothesize that *L. brevis* has anti-inflammatory activity due to its ability to inhibit, in particular on macrophagic cells, the activity of iNOS, indirectly causing a reduction in levels of inflammatory cytokines.

It was described the ability of some so-called protective microorganisms present in dental plaque, (for example *Streptococcus sanguinis*, Gram-positive, *Veillonella parvula*, Gram-negative, and *Eubacteria*), to suppress the growth of pathogenic bacteria. Therefore it would be advantageous to elim-
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Table 5 - Levels of nitrites/nitrates, PGE₂, cytokine and IgAs in controls and patients with periodontitis before (T0) treatment with L. brevis gums.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>T0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrites/nitrates (µM)</td>
<td>4.33 ± 4.89</td>
<td>38.86 ± 27.82*</td>
</tr>
<tr>
<td>IgAs (µg/ml)</td>
<td>885.62 ± 334.55</td>
<td>309.76 ± 51.29*</td>
</tr>
</tbody>
</table>

Data displayed as ± STD. *P < 0.05 mean.

Table 6 - Levels of nitrites/nitrates and IgAs in patients with gingivitis before (T0) and after (T1) treatment with L. brevis gums.

<table>
<thead>
<tr>
<th></th>
<th>T0</th>
<th>T1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrites/nitrates (µM)</td>
<td>38.86 ± 27.82</td>
<td>9.39 ± 6.72*</td>
</tr>
<tr>
<td>IgAs (µg/ml)</td>
<td>309.76 ± 51.29</td>
<td>466.51 ± 421.11</td>
</tr>
</tbody>
</table>

Data displayed as ± STD. *P < 0.05 mean.

administer harmful bacteria, encouraging the growth of beneficial microorganisms. We can not exclude that the administration of L. brevis, normally present in the oral flora and plaque, enabling an efficient competition with the same bacteria pathogens for periodontium for membership of adhesion sites on dental and epithelial surfaces.

From our research, as confirmed by clinical and laboratory investigation, results an effective anti-inflammatory action of arginina-deiminasi system, that some bacteria possessing. It is conceivable that the anti-inflammatory action of arginina-deiminasi system can also act in other inflammatory conditions, not necessarily where there is a bacterial component at the base, acting mainly through iNOS inhibition. This is the case for example of recurring aphthous stomatitis, multifactorial etiology disease, for which there isn't still a valid treatment without side effects.

Precisely for this inflammatory condition, we are implementing a therapeutic and research protocol, which aims to assess the efficacy of “arginine-deiminase” enzymatic system.

References


Figure 5 - Effect of gums with L. brevis on sensitivity to temperature and bleeding.

Figure 6 - Salivary IgA level of controls and patients with gingivitis marginals, before (T0) and after (T1) treatment with L. brevis gums.


