Effect of LongoVital treatment on development of periodontal disease in rats


LongoVital is a herbal tablet with documented immunostimulatory effects in man. In the present study the effect of LongoVital on development of periodontal disease was investigated in a rat model. Fifty-four conventional rats, 5 wk old, were distributed into the following groups: A) untreated, uninfected; B) untreated, infected with Actinomyces viscosus and Porphyromonas gingivalis wk 8; C) treated with LongoVital 80 mg x 3/wk, wk 5-14, uninfected; D) treated with LongoVital 80 mg x 3/wk, wk 5-14, infected with A. viscosus and P. gingivalis wk 8; E) treated with LongoVital 200 mg x 1 in wk 8 and 80 mg wk 9-14, uninfected; F) infected with A. viscosus and P. gingivalis wk 8, subsequently treated with LongoVital 200 mg x 1 in wk 8 and 80 mg wk 9-14. All animals were killed when they were 15 wk old, and periodontal bone support was assessed radiographically. Statistically significant bone loss was found in untreated, infected rats, as compared with untreated, uninfected rats. In LongoVital-treated animals, no significant difference was seen in bone level between infected and uninfected rats. These results indicate that LongoVital-treated rats were protected against periodontal bone loss caused by infection with A. viscosus and P. gingivalis. Furthermore, the protection seemed effective both when LongoVital was administered prophylactically and after exposure to periodontal pathogens. The active components of LongoVital, as well as the mechanisms responsible for the protection, remain obscure.

LongoVital (DK. Reg. No. 5178/75) is a tablet based on dried and ground herbs, including pumpkin seeds (Cucurbita pepo), peppermint (Mentha piperita), hawthorn (Crataegus spp.), rosemary leaves (Rosmarinus officinalis), paprika (Capsicum annuum), and milfoil (yarrow, Achillea millefolium), enriched with the nationally recommended doses of vitamins (1). In a recent double-blind, randomized, cross-over, 1-yr study, LongoVital was significantly better than placebo in preventing recurrent aphthous ulceration (RAU) (1). LongoVital treatment was also associated with a significant increase in CD4+ (T helper/inducer) lymphocytes and a minor increase in CD8+ (T suppressor/cytotoxic) lymphocytes in peripheral blood of RAU patients (2). No undesirable side-effects were seen in connection with daily use of LongoVital for 6 months (1).

The effect on CD4+ lymphocytes suggests a possible role for the drug in prevention or treatment of periodontal disease, since CD4+ cells seem implicated in the pathogenesis of this disease. In patients with periodontal disease, depressed ratios of CD4+/CD8+ lymphocytes have been reported in peripheral blood (3, 4) as well as locally in diseased periodontal sites (4, 5), indicating an immunoregulatory imbalance in relation to the disease. Furthermore, it is possible by adoptive transfer of cloned CD4+ cells to prevent development of experimentally induced periodontal disease in rats (6).

Accordingly, the present study was initiated in order to examine the effect of LongoVital treatment on development of periodontal disease in a rat model.

Material and methods

Experimental design – Fifty-four male and female specified-pathogen-free Wistar rats were included in the experiment. When the animals were 4 wk old, they were randomly distributed in six groups, A-F (Table 1). Based on the recommended daily intake (7) of the vitamins contained in LongoVital, a daily dose of 40 mg LongoVital per rat was estimated to be optimal. In order to minimize ex-
Experimental stress to the animals, this dosage was changed to 80 mg three times a week. LongoVital was dissolved in 1 ml drinking water and given to the animals by gastric feeding. Groups C and D were given this dosage from wk 5 until termination of the experiment. Three weeks after initiation of LongoVital treatment, groups B, D, and F were inoculated with a mixture of *Actinomyces viscosus* strain ATCC 19246 and *Porphyromonas gingivalis* (previously named *Bacteroides gingivalis*) strain 381. Approximately 10^6 living cells of each strain were dissolved in 1 ml carboxymethylecellulose and given to the rats by gastric feeding three times with 48-h intervals. Groups E and F received one dose of 200 mg LongoVital the day before the last inoculation and were treated like groups C and D during the subsequent weeks. Six weeks after the inoculation all animals were anesthetized with Immobilon, 1 ml of peripheral blood was obtained from axillary vessels, and the rats were killed by decapitation.

**Periodontal bone support** – Rat heads were autoclaved for 5 min and defleshed mechanically. Periodontal bone support was assessed radiographically, as described previously (8). In brief, the distances apex, deepest bony defect (AB) and apex, cusp tip (AC) were measured distal to mandibular first molars, and the ratio AB × 100/AC was calculated. Periodontal bone support was expressed as the mean of right and left ratios.

**Antibody assay** – Serum was isolated from the blood samples by centrifugation and examined for specific antibody activity against *P. gingivalis* and *A. viscosus* by enzyme-linked immunosorbent assay (ELISA). Ninety-six-well microtiter plates (Nunc, Roskilde, Denmark) were coated with whole bacterial cells, 10^5 cells/ml in phosphate buffer (pH 7.2), 50 µl/well, and incubated overnight at 5°C. Then the wells were blocked for 1 h with 200 µl coating buffer containing 1% bovine serum albumin. This step and the subsequent steps were performed at room temperature. After washing three times with phosphate buffer (pH 7.2) containing 0.15% Tween 20, 50 µl rat serum diluted 1:10 in washing buffer was added to each well and incubated for 2 h. After washing four times, the wells were incubated for 1 h with horseradish peroxidase conjugated monoclonal mouse antirat kappa chain antibody (Zymed Laboratories, San Francisco, CA, USA), diluted 1:250 in washing buffer. After washing five times, 100 µl color buffer (phosphate buffer with citric acid, pH 5.0), containing 0.05% ortho-phenylene-diamine and 0.01% H₂O₂, was added as substrate for peroxidase bound to the plate and incubated for 45 min. The enzyme substrate reaction was stopped by adding 50 µl 1.0 mol/l NaOH to the wells, and the optical density (OD) of each well was determined at 450 nm by an optical reader (TIM-200, Intermed, Tecnunc, Roskilde, Denmark). A control well, subjected to all steps except the addition of rat serum, was designated as blank, and the OD value of this well was automatically subtracted from the OD values of all other wells. All serum samples were run in four wells, and the mean OD was calculated for each sample.

**Statistics** – The differences between infected and uninfected groups within each treatment group were tested by the Mann-Whitney U test. Differences between the various treatments within infected and uninfected groups were tested by the Kruskall-Wallis test. For further analysis the material was subjected to a general linear model (GLM) procedure and Duncan’s multiple range test. In all statistical tests the 5% level of significance was chosen.

**Results**

**Periodontal bone support** – Among animals not treated with LongoVital, infected rats had significantly lower periodontal bone support than uninfected rats (Table 2). In the groups treated with LongoVital, the mean bone support was also lower in infected rats, but in these cases the differences were not significant. No significant differences were found between the three treatment modalities in either infected or uninfected animals.

**Serum antibodies** – Some antibodies reactive to *P. gingivalis* antigens were evident in all animal

Table 1

<table>
<thead>
<tr>
<th>n</th>
<th>LongoVital treatment</th>
<th>Infection</th>
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<tbody>
<tr>
<td>A 10</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>B 7</td>
<td>None</td>
<td><em>A. viscosus</em> + <em>P. gingivalis</em>, wk 8</td>
</tr>
<tr>
<td>C 8</td>
<td>Low dose⁶, wk 5-14</td>
<td>None</td>
</tr>
<tr>
<td>D 10</td>
<td>Low dose⁶, wk 5-14</td>
<td><em>A. viscosus</em> + <em>P. gingivalis</em>, wk 8</td>
</tr>
<tr>
<td>E 9</td>
<td>High dose⁷, wk 8</td>
<td>None</td>
</tr>
<tr>
<td>F 10</td>
<td>High dose⁷, wk 9-14</td>
<td><em>A. viscosus</em> + <em>P. gingivalis</em>, Low dose⁷, wk 9-14, wk 8</td>
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⁶ 80 mg × 3/wk. ⁷ 200 mg × 1.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Uninfected</th>
<th>LongoVital low dose⁶</th>
<th>LongoVital high dose⁶</th>
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</thead>
<tbody>
<tr>
<td>Uninfected</td>
<td>56.84 ± 0.76</td>
<td>55.81 ± 0.75</td>
<td>56.17 ± 0.95</td>
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<tr>
<td>Infected</td>
<td>53.54 ± 1.18</td>
<td>55.51 ± 0.93</td>
<td>55.34 ± 0.46</td>
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| p < 0.05 | NS | NS |

⁶ 80 mg × 3/wk, start 3 wk before inoculation.

⁷ 200 mg 3 days after inoculation, subsequently 80 mg × 3/wk.
Table 3

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>LongoVital low dose</th>
<th>LongoVital high dose</th>
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<tbody>
<tr>
<td>Uninfected</td>
<td>98.71 ± 11.21</td>
<td>84.96 ± 13.72</td>
<td>103.27 ± 19.20 NS</td>
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<tr>
<td>Infected</td>
<td>189.55 ± 69.31</td>
<td>148.45 ± 32.31</td>
<td>206.25 ± 40.86 NS</td>
</tr>
<tr>
<td></td>
<td>NS</td>
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<td>NS</td>
</tr>
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<sup>a</sup> 80 mg x 3/wk, start 3 wk before inoculation.
<sup>b</sup> 200 mg 3 days after inoculation, subsequently 80 mg x 3/wk.

groups, but, in general, the response was stronger in infected animals (Table 3). By the Mann-Whitney test, no significant differences were found, probably because of considerable variation within the groups. However, when considering all animals at one time by GLM and Duncan’s test, a significant difference between infected and uninfected rats was revealed. No difference was found between the three treatment modalities. The antibody response to *A. viscosus* antigens was quite high in all animal groups, and no significant differences were found (Table 4).

Discussion

The present study confirmed previous observations that mixed infections with *A. viscosus* and *P. gingivalis* can induce measurable periodontal bone loss in conventional rats (9, 10). Both *A. viscosus* (11–13) and *P. gingivalis* (14, 15) can cause periodontal bone loss by monoinfection in rats, and since a high disposition to cohesion exists between the two strains (16), colonization may be improved by inoculating them together.

Interestingly, no significant bone loss was seen in animals treated with LongoVital, indicating that the treatment had exerted protection against this effect of the periodontal infection. Protection was found in rats that were given LongoVital prophylactically, as well as in animals that received the first dose 3 days after first inoculation with periodontopathic bacteria. This finding is in line with previous case reports which indicated that a high dose (3-5 times recommended daily dose) may relieve symptoms of acute infections in human beings (17).

The constituents responsible for this protective effect of LongoVital are presently unknown. It is generally recognized that inadequate nutrition, most notably deficiencies in protein and vitamins, may render the host more susceptible to periodontal disease (18), although extensive epidemiologic studies have revealed only very weak correlations between periodontal status and deficiencies in vitamin A, ascorbic acid, thiamine, and riboflavin (19). Furthermore, all rats in the present study had access to standard food pellets containing recommended amounts of all vitamins, and it is therefore unlikely that the control rats would be deficient in any of these nutrients.

The effect is more likely to be due to the herbs included in LongoVital, since some of them exert documented or alleged antiinflammatory or antibiotic effects. Rosemary has been used in treatment of bacterial infections of the urinary and female genital systems, and peppermint and paprika both contain antinfective substances (17, 20, 21). Milfoil, like the closely related chamomile, also contains definite antiinflammatory compounds, namely chamazulene and *z*-bisabolol (20, 21). These volatile oil components are poorly exploited when milfoil is ingested in tea, whereas whole plant preparations, such as LongoVital, are more effective (21).

The mechanisms by which protection is provided were not investigated in the present study. As mentioned above, an increase in CD4+ cells may be suspected in treated animals, but, if this occurred, it did not influence the production of serum antibody against the inoculated organisms (Tables 3 and 4). Future studies will have to investigate possible effects of LongoVital, or single components thereof, on lymphocyte populations, cytokine production, and other elements of the immune system, as well as on periodontal microorganisms. Furthermore, investigations of possible effects on periodontal disease in man seem warranted.

In conclusion, the present study indicates that ingestion of LongoVital, a harmless herbal preparation with documented immunostimulatory effects, can interfere with the progression of periodontal disease in rats. At present the active components as well as the mechanisms behind this effect are speculative.

Table 4

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<tr>
<th></th>
<th>Untreated</th>
<th>LongoVital low dose</th>
<th>LongoVital high dose</th>
</tr>
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<tbody>
<tr>
<td>Uninfected</td>
<td>120.18 ± 21.14</td>
<td>178.65 ± 49.78</td>
<td>130.71 ± 33.27 NS</td>
</tr>
<tr>
<td>Infected</td>
<td>129.50 ± 29.96</td>
<td>175.16 ± 51.13</td>
<td>138.21 ± 14.08 NS</td>
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<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<sup>a</sup> 80 mg x 3/wk, start 3 wk before inoculation.
<sup>b</sup> 200 mg 3 days after inoculation, subsequently 80 mg x 3/wk.

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References


