Daily Intake of Heat-killed *Lactobacillus plantarum* L-137 Decreases the Probing Depth in Patients Undergoing Supportive Periodontal Therapy

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Purpose: Heat-killed *Lactobacillus plantarum* L-137 (HK L-137) has been shown to activate innate and acquired immunity in humans. The aim of this randomised, double-blind, placebo-controlled clinical trial was to examine the effects of the oral administration of HK L-137 on the outcome of periodontal therapy.

Materials and Methods: Thirty-nine patients undergoing supportive periodontal therapy (SPT) were randomly assigned to receive a capsule containing 10 mg of HK L-137 or a placebo capsule daily for 12 weeks. Nineteen patients in the experimental group and 17 patients in the control group were followed-up. Clinical parameters, including plaque index (PI), gingival index (GI), bleeding on probing (BOP), and probing depth (PD) were scored at baseline and weeks 4, 8 and 12 prior to prophylaxis in conjunction with regular SPT visits.

Results: BOP and the number of teeth or sites with PD ≥ 4 mm were significantly reduced in both groups by a successive SPT programme, while there was significantly greater PD reduction ($p < 0.05$) at teeth with site(s) with PD ≥ 4 mm at baseline in the experimental group than in the control group at week 12.

Conclusion: These clinical findings suggest that daily HK L-137 intake can decrease the depth of periodontal pockets in patients undergoing supportive periodontal therapy.

Key words: dental plaque, gingivitis, *Lactobacillus plantarum*, probing depth, randomised clinical trial

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The primary aetiology of periodontal disease is the interaction between pathogenic microorganisms and the host's defensive mechanisms. Dental plaque is considered to be the primary aetiological factor for periodontitis, and there is considerable scientific evidence supporting the importance of its removal in maintaining a healthy periodontium.[7,12,14] However, it is well established that shortcomings in subgingival instrumentation may limit the clinical outcome of periodontal therapy. Periodontal pathogens persist after mechanical debridement because of their ability to invade soft tissues;[5,32] also, they may be located in anatomic 'hideouts,' such as dentinal tubuli, furcations, or deep infrabony defects with difficult access, where they are protected from thorough instrumentation.[1,8,30] Pathogenic bacteria also reside on other oral, nonperiodontal surfaces, such as the mucosa, tongue and tonsils, and may translocate to and reinfect periodontal sites after mechanical instrumentation.[27] Furthermore, some

patients exhibit a higher susceptibility to periodontal disease and may respond less favourably to well-executed mechanical treatment regimens.[31]

The use of probiotics has attracted the interest of the scientific community as a new alternative adjunctive treatment for periodontitis. Probiotics are originally defined as living microorganisms that exert health benefits beyond inherent basic nutrition when ingested in certain numbers.[13,19,21] A study comparing the composition of oral lactoflora between healthy subjects and subjects with chronic periodontitis observed a higher prevalence of homofermentative lactobacilli, particularly *L. gasseri*, in healthy subjects.[20] Oral lactobacilli have been shown to suppress the growth of periodontal pathogens both in vitro[20,34] and in vivo, and to improve several symptoms of chronic periodontitis.[39,40] The lactic acid produced by lactic acid bacteria through carbohydrate fermentation is reported to be one of the factors generating a low pH and inhibiting the growth of anaerobic bacteria.[35]

Heat-killed *Lactobacillus plantarum* L-137 (HK L-137), a strain isolated from fermented food, is a potent inducer of IL-12, which leads to a T-helper-1 (Th1) type immune response and subsequent antiallergic or antitumor effects in mouse models.[10,17,24-26] It has also been demonstrated that oral administration of HK L-137 enhances protection against influenza virus infection by stimulation of type I IFN production in mice.[23] Furthermore, enhanced Th1-related immune functions and increased production of type I IFN have been observed after intake of HK L-137 in humans.[2,16,18] The host immune response has been reported to play a role in periodontal disease progression, including periodontal infection and alveolar bone resorption. As described above, the effects of probiotics on chronic periodontitis are well accepted, but the effects of an immunopotentiator such as HK L-137 on chronic periodontitis are poorly understood.

The present study was performed to evaluate the efficacy of daily oral administration of HK L-137 on periodontal status following supportive periodontal therapy (SPT).

MATERIALS AND METHODS

Preparation of heat-killed L. plantarum L-137

LP20 (House Wellness Foods; Hyogo, Japan) containing 20% HK L-137 and 80% dextrin was used in the present study. HK L-137 for LP20 was prepared according to a previously described method.[24]

Subjects

Patients with chronic periodontitis participating in a supportive periodontal therapy (SPT) programme were recruited from August 2012 to April 2013 and were assessed for eligibility to take part in the present study. The inclusion criteria were: being an SPT patient with chronic periodontitis, having one or more initial periodontal pockets ≥ 4 mm and having completed active treatment for chronic periodontitis. Exclusion criteria included allergy to milk or soy, treatment with a prescribed antimicrobial agent within the previous month, expectant mothers, poor compliance with the clinical trial guidelines, and being an inappropriate subject as judged by the investigator. Among 40 SPT patients with chronic periodontitis recruited, thirty-nine (24 women and 15 men; mean age 66.2 years) were eligible and randomly assigned to the control or experimental group (Fig 1). Sample size was determined based on previous studies of the effects of antioxidant supplementation on clinical effects, especially probing depth (PD).[4] Considering a standard deviation of 0.23 mm and a difference between the experimental and control group of 0.22 mm, it was calculated that 18 patients were needed in each group to provide 80% power and a type I error of 5%. Forty patients were intially recruited to take an estimated 10% dropout rate over the study period into account. This study was approved by the Ethics Committee of Kanagawa Dental University in accordance with the Helsinki Declaration of 1975 as revised in 2008. The procedures were fully explained to the subjects, and written informed consent was obtained from each subject before the beginning of the study.

Experimental design

A total of thirty-nine SPT patients who completed active treatment for chronic periodontitis followed by SPT every 4 weeks were enrolled in a randomised, double-blind, placebo-controlled, paral-

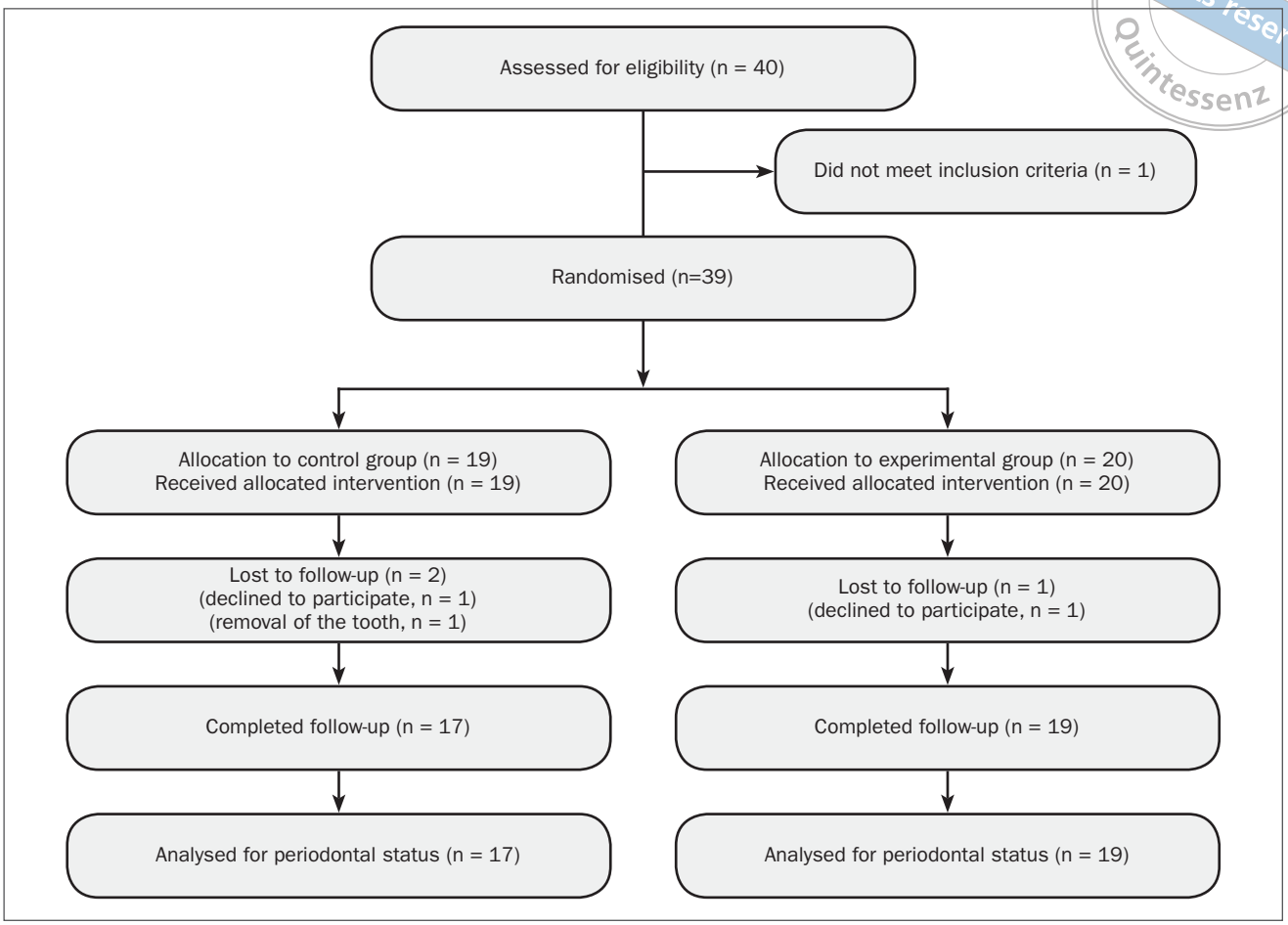

Fig 1 Flowchart of subjects.

lel-design study. The subjects were randomly assigned to two groups using a sequence of numbered, sealed envelopes that each contained either LP20 or dextrin, assigned in a computer-randomised manner. After the assignment, the subjects consumed a hard gelatin capsule containing either 50 mg of LP20 (1 capsule/day) or a matching placebo capsule in which dextrin was substituted for LP20 for 12 weeks. Cellulose was used as the excipient for both capsules. The SPT programmes and clinical examinations were performed at baseline, 4, 8 and 12 weeks after the start of dietary intervention. Each SPT visit consisted of clinical examinations including the full-mouth plaque index (PI), gingival index (GI), bleeding on probing (BOP) and PD, the reinforcement of oral hygiene measures, supragingival and subgingival scaling, and polishing and occlusal adjustment when needed to increase patient comfort. The study was conducted at the hospital of Kanagawa Dental University (Yokosuka, Japan) from August 2012 to July 2013.

Clinical measurements

The full-mouth GI[22] and full-mouth PI[33] were calculated. BOP and PD were measured using a Williams probe (Hu-Friedy; Chicago, IL, USA) at six sites per tooth. BOP was recorded as present or absent at six sites per tooth and the full-mouth prevalence (%) of BOP was calculated. Other information, such as age, gender and number of teeth, was collected before the start of dietary intervention.

Statistical analyses

Data analyses were performed with a statistics software programme (SPSS II 11.0.1; Tokyo, Japan). The baseline values were compared between the two groups using Pearson's chi-square test for gender or the unpaired t-test for the other parameters. The mean clinical parameters were analysed by repeated measure ANOVA, followed by comparisons between

Table 1 Demographic and clinical parameters for control and experimental groups at baseline

	Control group (n=17)	Experimental group (n=19)
	Mean (SD)	
Age (years)	67.0 (8.1)	68.2 (9.7)
Gender (male: female)	7: 10	6: 13
Teeth (N)	25.1 (3.1)	24.4 (3.9)
Plaque Index, PI	0.22 (0.23)	0.20 (0.15)
Gingival Index, GI	0.13 (0.13)	0.16 (0.16)
Bleeding on probing, BOP (%)	11.7 (8.3)	9.5 (7.8)
Probing depth, PD (mm)	2.17 (0.36)	2.21 (0.32)
Number of teeth with PD ≥ 4 mm	4.1 (3.5)	3.5 (2.2)
Number of sites with PD ≥ 4 mm	7.5 (8.2)	6.2 (5.5)

The baseline values between two groups were compared using Pearson's χ^2 test for gender or the unpaired t-test for the other parameters.

the two groups at each time point using the unpaired t-test. Changes from baseline in the clinical parameters and number of teeth and sites with PD ≥ 4 mm were analysed by two-way ANOVA, followed by comparisons between the two groups at each time point using the unpaired t-test. The intergroup differences were considered significant at $p < 0.05$.

RESULTS

Baseline characteristics

Before completing the study, 2 subjects in the control group dropped out – one declined to participate and one had a tooth removed – and 1 subject in the experimental group also declined to participate. A total of 36 subjects completed the present study, and were included in the statistical analysis (Fig 1). Baseline characteristics in demographic and clinical parameters did not differ between the two groups (Table 1).

Change of mean clinical parameters

BOP, the number of teeth with site(s) having PD ≥ 4 mm, and the number of sites with PD ≥ 4 mm were significantly reduced in both groups by the SPT programme, but there were no differences in any of the clinical parameters between the two groups (Table 2).

Change of mean BOP and PD in teeth with PD ≥ 4 mm

Next, the improvement of PD and BOP in teeth with site(s) with probing depths 4 mm or deeper at baseline was compared between the two groups (Table 3). The mean PD with initial PD ≥ 4 mm was significantly reduced in both groups by a subsequent SPT programme. Moreover, there was significantly greater PD reduction ($p < 0.05$) in teeth with an initial PD ≥ 4 mm in the experimental group than in the control group at week 12.

DISCUSSION

In the present study, we examined the effects of the daily intake of HK L-137 on the outcomes of periodontal therapy. It was demonstrated that the PD of periodontal pockets greater than 4 mm deep at baseline showed significantly greater reduction in the experimental group than in the control group among individuals undergoing SPT.

SPT programmes typically include an update of patient information, a clinical evaluation of the dentition and periodontium, removal of dental biofilm by plaque control, supra- and subgingival scaling and root planing, and reinforcement of oral hygiene instruction. Clinical parameters such as PD, BOP, GI and PI as well as CAL (clinical attachment level) are used to evaluate the result achieved after SPT. The CAL is the distance from the cementoenamel junction (CEJ) to the bottom of periodontal pocket.

Table 2 The overall change of mean clinical parameters

		Mean (SD)				Repeated measure ANOVA, p-value		
		Baseline	4 weeks	8 weeks	12 weeks	Intervention	Time periods	Interaction
PI	Control group	0.22 (0.23)	0.22 (0.21)	0.19 (0.20)	0.17 (0.13)	0.705	0.100	0.843
	Experimental group	0.20 (0.15)	0.18 (0.17)	0.17 (0.13)	0.16 (0.18)			
GI	Control group	0.13 (0.13)	0.13 (0.13)	0.14 (0.15)	0.12 (0.15)	0.800	0.872	0.565
	Experimental group	0.16 (0.16)	0.13 (0.11)	0.13 (0.13)	0.15 (0.16)			
BOP (%)	Control group	11.7 (8.3)	8.8 (7.4)	9.8 (6.5)	8.0 (6.8)	0.952	0.022	0.112
	Experimental group	9.5 (7.8)	9.7 (9.9)	9.6 (9.7)	8.8 (9.7)			
PD (mm)	Control group	2.17 (0.36)	2.15 (0.32)	2.14 (0.35)	2.15 (0.37)	0.932	0.288	0.569
	Experimental group	2.21 (0.32)	2.13 (0.38)	2.18 (0.39)	2.12 (0.36)			
Changes from baseline (mm)	Control group		-0.02 (0.19)	-0.03 (0.22)	-0.02 (0.22)	0.262	0.768	0.684
	Experimental group		-0.08 (0.20)	-0.03 (0.20)	-0.09 (0.15)			
Number of teeth with PD ≥ 4 mm	Control group	4.1 (3.5)	2.9 (2.7)	3.4 (3.3)	3.4 (3.1)	0.499	0.001	0.362
	Experimental group	3.5 (2.2)	2.8 (2.4)	2.7 (2.4)	2.4 (2.6)			
Changes from baseline	Control group		-1.2 (1.7)	-0.6 (1.6)	-0.7 (1.1)	0.955	0.819	0.437
	Experimental group		-0.7 (1.4)	-0.8 (1.6)	-1.1 (1.5)			
Number of sites with PD ≥ 4 mm	Control group	7.5 (8.2)	4.9 (5.2)	5.6 (6.0)	5.9 (7.1)	0.720	0.006	0.174
	Experimental group	6.2 (5.5)	5.4 (6.7)	5.2 (7.5)	4.2 (6.5)			
Changes from baseline	Control group		-2.6 (4.2)	-1.9 (3.3)	-1.6 (2.4)	0.176	0.883	0.302
	Experimental group		-0.7 (2.9)	-1.0 (3.2)	-1.9 (2.3)			

Control group: n = 17; experimental group: n = 19. Significant differences in the clinical parameters between the two groups over the time periods were evaluated by repeated measure ANOVA, followed by comparison at each time point by the unpaired t-test. Significant differences in changes from baseline between the two groups over the time periods were evaluated by two-way ANOVA, followed by comparison at each time point by the unpaired t-test.

Because the CEJ is a fixed point that does not change throughout life, measurement of CAL has become a standard for diagnosing and monitoring periodontal disease. The Michigan studies[28,29] and others[3,6,9] have shown that nonsurgical treatment such as scaling and root planing lead not only to PD reductions but also to CAL gains from baseline levels in patients with chronic periodontal diseases. A

Table 3 The change of mean BOP and PD in teeth with PD ≥ 4 mm

		Mean (SD)				Repeated measure ANOVA, p-value		
		Baseline	4 weeks	8 weeks	12 weeks	Intervention	Time periods	Interaction
BOP (%)	Control group	28.6 (12.5)	18.2 (12.0)	22.3 (11.8)	15.5 (11.2)	0.893	0.002	0.300
	Experimental group	24.8 (16.8)	19.8 (17.6)	21.9 (22.7)	20.5 (17.4)			
PD (mm)	Control group	3.00 (0.29)	2.80 (0.40)	2.86 (0.35)	2.87 (0.30)	0.989	0.001	0.313
	Experimental group	3.08 (0.34)	2.84 (0.47)	2.89 (0.53)	2.72 (0.43)			
Changes from baseline (mm)	Control group		-0.20 (0.56)	-0.14 (0.35)	-0.13 (0.29)	0.148	0.611	0.470
	Experimental group		-0.24 (0.34)	-0.19 (0.38)	-0.36 (0.23)*			

Control group: n = 17; experimental group: n = 19. Significant differences in the clinical parameters between the two groups over the time periods were evaluated by repeated measure ANOVA, followed by comparison at each time point by the unpaired t-test. Significant differences in changes from baseline between the two groups over the time periods were evaluated by two-way ANOVA, followed by comparison at each time point by the unpaired t-test. *Different from the control group at that time, $p < 0.05$.

systematic review[15] has also illustrated that the improvement of PD correlates with that of CAL in moderately deep (4–6 mm) and deep (>6 mm) pockets more strongly than in unremarkable pockets (1–3 mm) after 12 months of SPT. Since patients with chronic periodontitis having one or more initial periodontal pockets ≥ 4 mm during the SPT programme were recruited in this study, the authors find that the combination of clinical parameters such as PD, BOP, GI and PI without CAL are sufficiently effective for evaluating the results achieved after dietary intervention.

In previous studies,[2,16,18] we reported that healthy subjects with a daily intake of HK L-137 showed sustained increases in Th1-type immune responses from 4 weeks, such as concanavalin-A–induced proliferation of peripheral blood mononuclear cells, percentages of IFN-γ and IL-4-producing CD4+ T cells (Th1:Th2 ratio), or serum IFN-β concentration, whereas that the health-related QoL or the incidence of upper respiratory tract infection improved from 8 weeks after the start of dietary intervention. In this study, PD reduction in teeth with PD ≥ 4 mm at baseline was significantly greater in the experimental group than in the control group at 12 weeks (Table 3). It is possible that the continuous intake of HK L-137 for more than 4 to 8 weeks is also required for suppressing the progression of chronic periodotitis.

The precise mechanism of how intake of HK L-137 promoted PD reduction in moderate or deep pockets in patients during a SPT programme is unclear. The immune response against periodontopathic bacteria is regulated by the balance between cytokines produced by Th1 and Th2 cells.[11] Various inflammatory cytokines have been also shown to play a role in osteoclast genesis, including IFN-α, IFN-β and IFN-γ, and the negative effect of IFN-γ on osteoclast genesis has been the most extensively characterised among these inflammatory cytokines.[36-38] HK L-137 is a potent inducer of IL-12, which leads to a Th1-type immune response through IFN-γ induction;[24-26] it has been reported that daily intake of HK L-137 enhanced Th1-related immune functions and IFN-β production in healthy subjects.[2,16,18] It is possible that the PD reduction in teeth with PD > 4 mm is related in some way to the enhancement of IFN-β and IFN-γ production in subjects who consume HK L-137. Further studies are needed to reveal those mechanisms.

CONCLUSION

The results of the present study indicate that the daily intake of HK L-137 in patients with chronic periodontitis treated and enrolled in an SPT programme may lead to additional PD reduction at sites with PD ≥ 4 mm.

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