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A randomized, single-blind, parallel-group clinical study to evaluate the effect of soluble β-1,3/1,6-glucan on experimental gingivitis in man

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Abstract

Background: Gingivitis is an inflammatory disorder of the periodontium induced by dental plaque bacteria. Soluble β-1,3/1,6-glucan (SBG) is known to enhance infection defense by preventing excessive inflammatory responses caused by bacterial endotoxins.

Aim: The aim of the present study was to investigate the effect of SBG on experimental gingivitis in man.

Material and Methods: Experimental gingivitis was induced over a period of 24 days in 30 healthy volunteers who were simultaneously treated with SBG. Two groups ($n = 10$/group) rinsed twice daily with an SBG mouthwash that was either swallowed or expectorated. A third group ($n = 10$) received a water rinse as a control. Plaque index (Pl.I), gingival index (GI), and amount of gingival crevicular fluid (GCF) were assessed at baseline and at six times during the study.

Results: The results showed that in the SBG groups, GCF decreased significantly during the study. The swallow group experienced a significant increase in GCF during the first week. The control group followed the expected pattern of experimental gingivitis, with a significant increase in the gingival fluid secretion during the test period. There was a significant increase in GI and Pl.I during the study for all groups, with no significant differences between them. No adverse effects of SBG were recorded.

Conclusions: In this 24-day experimental gingivitis study of subjects who used either a SBG or a control mouthrinse: (1) all subjects had increased plaque and gingivitis, (2) GCF increased in control-rinse subjects and GCF decreased in SBG-rinse subjects. The only statistically significant difference between the SBG-rinse and control-rinse subjects was an increase in GCF at day 7 for subjects who rinsed and swallowed SBG.

Key words: gingivitis; glucan; RCT

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Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests.

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Chronic gingivitis is generally defined as a reversible, non-specific bacterial infection in the gingival soft tissues (Page & Schroeder 1982, Schätzle et al. 2003). The condition is extremely common; symptoms range from clinically unnoticeable to severe with oedema, change in colour, provoked or spontaneous bleeding and possibly pain or discomfort. Gingivitis is reversible if the biofilm, commonly referred to as dental plaque, is effectively removed (Løe et al. 1965). Consequently, mechanical plaque removal has been the predominant method for treatment of gingivitis.

β-1,3/1,6-glucan

β-1,3/1,6-glucans are known to activate immune cells and to enhance infection defense (Seljelid 1990, Robertsen et al. 1994, Bohn & BeMiller 1995, Franz et al. 1995, Engstad & Robertsen 1995, Williams et al. 1996, 1997, Misaki & Kakuta 1997) and at the same time to down-regulate inflammation caused by infectious microorganisms (Rice et al. 2005). β-1,3/1,6-glucans also aid in wound healing by stimulating tissue regeneration (Williams & Bowder 1987, Delatte et al. 2001).

Soluble β-1,3/1,6-glucan (SBG) modulates immune mechanisms by interacting with innate immune cells such as macrophages, dendritic cells, granulocytes and natural killer cells (Brown & Gordon 2005). These cells are equipped with surface receptors called pattern recognition receptors (PRRs), which discriminate between different ''pathogen-associated molecular patterns'' (PAMPs). The PRRs embrace receptors such as toll-like receptors (TLRs), the dectin-1 receptor, the mannose receptor, and also the β-glucan binding part of complement receptor 3. The most pronounced PRRs are the TLRs, of which 11 have so far been characterized and numbered TLR 1–TLR 11. Bacterial lipopeptides and peptidoglycans interact with TLR 2, bacterial lipopolysaccharides (LPS) reacts with TLR 4, bacterial DNA reacts with TLR 9, and β-1,3/1,6-glucans, either alone or in concert with dectin-1, interact with TLR 2 and/or TLR 6 (Brown 2006, Gersuk et al. 2006, Uematsu & Akira 2006). The pattern of signal molecules (cytokines) induced by PAMPs differs greatly depending on the receptor involved. For example, engagement of TLR 4 typically elicits the production of cytokines such as TNF-α, which induces inflammation and fever, whereas the engagement of TLR 2 and TLR 3 induces interferon-γ (IFN) production. Furthermore, the response by one PRR can be modulated by the simultaneous or subsequent engagement of other receptors, as in the case where β-1,3/1,6-glucans modulate the immune response to LPS. This explains why β-1,3/1,6-glucans contribute to counterbalance the inflammatory response induced by LPS and counteract sepsis, while enhancing infection defence (Rice et al. 2005, Sandvik et al. 2007). Of particular relevance for use of SBG in gingivitis prophylaxis or treatment is the fact that it acts via the mucosal immune system (Raa et al. 2000) and counteracts inflammations even if they are induced experimentally by injection.

In summary, SBG would be expected to enhance the body's innate and non-specific immune defense mechanisms in the periodontal tissues and modulate gingivitis by one of two mechanisms: (1) increasing gingivitis within protective levels and/or, (2) decreasing levels of inflammation, clinically expressed as gingivitis. SBG has been used successfully against different infectious diseases in animals and fish (Robertsen et al. 1990, Dritz et al. 1995). Studies in humans have shown that the product can be used safely and has no adverse side-effects (Lehne et al. 2006). Moreover, preliminary studies by Breivik et al. (2005) showed that SBG from yeast inhibited experimental periodontal disease in a rat model. SBG also functions when applied on mucosal surfaces (Raa et al. 2000) and β-glucans aid wound healing by stimulating tissue regeneration (Williams & Bowder 1987, Delatte et al. 2001).

SBG has never been tested against any of the periodontal diseases in humans. SBG is known to activate immune cells in mucosal tissues, thereby enhancing infection defense. Consequently, SBG may have an impact on the development of gingivitis and/or periodontitis.

The aim of the present study was, therefore, to test the clinical effect of a newly developed SBG on experimentally induced gingivitis in humans. A secondary objective was to monitor adverse events, vital signs, haematology and biochemical parameters in subjects who rinse with SBG. The protocol was approved by the Norwegian Medicines Agency and the Regional Committee for Medical Research Ethics (Protocol Code; SBG-1-09, Reference: S-04066). The study was also monitored by an independent clinical research organization (SCR – Scandinavian Clinical Research, Lillestrøm, Norway).

Material and Methods

A group of volunteering dental, medical and dental hygiene students was invited to participate in a seminar on periodontitis/gingivitis that included chemical prevention and treatment of these diseases. A special section was allocated to the molecular, pharmacological and biological effect of SBG, ending up with information about the planned study and an invitation to participate. Following this seminar, students were given the opportunity to give their written informed consent and to be accepted for screening for the study. All subjects were screened before randomization using the following eligibility criteria.

Inclusion criteria:

- Medically healthy, non-smoking subjects of both genders, 18–35 years of age.
- Absence of gingivitis.
- Presence of three of the following four teeth in both maxillary quadrants: canine, first bicuspid, second bicuspid, first molar.

Exclusion criteria:

- Pregnancy, lactation or absence of adequate contraception for fertile women.
- Any chronic disease.
- Clinical signs or symptoms of acute infection in the oral cavity.
- Any prescription or non-prescription systemic or topical medication (except oral contraceptives), administered within 1 week before start of study.
- Haematological and clinical/chemical parameters judged as unacceptable by the investigator.
- Use of systemic antibiotics the last 3 months before start of study.
- History of alcohol or drug abuse.
- Participation in other clinical studies in the last 4 weeks.

The study period lasted 24 days during which no special religious or ethnic feasts or events jeopardized the collec-

tive behaviour of the study population. All participants signed a written, informed consent, and all information, administration and data collection were performed at the Department of Periodontology, IKO, Faculty of Dentistry, University of Oslo, Norway.

After screening for eligibility criteria, 24 women and 6 men, ranging from 18 to 35 years of age (mean age 23.4), were randomized using a computer program into three study groups of 10 participants per group as follows:

(1) *SBG rinse and swallow*: 15 ml oral rinse twice daily for 2 min. and then swallowed.
(2) *SBG rinse and expectorate*: 15 ml oral rinse twice daily for 2 min. and then expectorated.
(3) *Control rinse*: 15 ml water oral rinse twice daily for 2 min. and then expectorated.

The SBG oral rinse consisted of 15 ml SBG as 1.5% w/v solution in water (Biotec Pharmacon ASA, Tromsø, Norway).

Experimental gingivitis (Løe et al. 1965) was induced in the participants by allowing plaque accumulation in the upper right quadrant for 24 days. This was achieved by covering this area of the mouth with an individually designed mouthguard that was fixed to the teeth only during daily tooth cleaning. The upper, left quadrant was used to assess the same parameters on contra-lateral teeth where normal oral hygiene was performed (with no mouthguard). Treatment effect and safety variables were assessed at screening and at days 0, 7, 14, 17, 21 and 24 using plaque index (Pl.I), gingival index (GI) (Løe 1967) and gingival crevicular fluid (GCF) flow from the mesio-buccal surfaces of all test and control teeth. GCF produced during gingivitis is correlated with the degree of inflammation during gingivitis and is considered to be sensitive to small variations in inflammation (Lamster et al. 1985). GCF was quantified by a Periotron 8000® gingival fluid meter (Oraflow Inc., Plainview, NY, USA). Test teeth were 13–16 and control teeth were 23–26 (Fédération Dentaire Internationale). GCF was collected and measured according to the manufacturer's instructions (Oraflow Inc.). In each participant, the experimental area was carefully dried with a gentle blast of air and kept dry with a cotton roll placed in the maxillary vestibule during the measuring procedures. A PerioPaper strip (Oraflow Inc.) was inserted until resistance was felt, and left for 30 s in the gingival crevice on the mesio-buccal surface of the selected teeth, and then discarded. A new PerioPaper strip was then inserted and the procedure was immediately repeated. To assess the GCF flow rate, quantification in the Periotron 8000® was performed immediately after collecting the fluid. Sampling time, time between sampling and time from sampling to reading was thoroughly rehearsed before starting the experiment and a stopwatch was used during the investigation in order to standardize all time periods. The same procedure was performed for all test and control teeth. Because variations in hormone levels are known to influence the production of GCF, the start and end dates of the last menstruation cycle for all the female participants were recorded at the last visit on day 24.

Safety of the treatment medication was evaluated throughout the study period on the basis of vital signs (blood pressure, heart rate) and by monitoring haematological values (erythrocytes, haemoglobin (Hb), haematocrit (Hct), white blood cell count, platelet count, erythrocyte sedimentation rate (E-SR), creatinine, alkaline phosphatase, ASAT, ALAT, total bilirubin, γ-GT and CRP and biochemical standard values of urine samples (pH, protein, glucose, ketone bodies, haemoglobin, WBC, nitrite). A pregnancy test was carried out on all female participants at the first visit. The extensive blood and urine work were in compliance with Norwegian regulations. A team of four clinical researchers was trained in obtaining informed consent, entering subjects into the study, administering the questionnaire, and clinical monitoring at all visits. Each researcher had his/her place in the organization and was thoroughly calibrated before the start of the study. Between appointments, study subjects were in touch with the research leader by SMS messaging and e-mail. All participants received written confirmation of their next appointment at the end of each appointment, e-mails confirming day appointments, and SMS reminder messages the evening before and 1 h before the scheduled appointments. The success of this close follow-up was evidenced by the fact that no appointments were missed by any study subject. The test product, dose and mode of administration were explained to all study participants at the baseline appointment. All participants received the same information, and upon leaving the room, they were asked to open the box with medication out of view of the project leader. The box contained information on how to use the test/control rinse and expectorate or swallow. Preceding every examination, all participants were interviewed about compliance and questioned about any special observations and/or complaints. Therefore, compliance with the protocol was re-inforced regularly.

The project leader and the data collection team were totally blinded to the group assignment. Obviously, participants themselves knew who swallowed after using the test/control or who only rinsed with the test/control solution. In the interview preceding screening, all students reported that the taste of the solution was like water, but some reported a smoother feeling in the mouth. We do not know whether this related to the test or the control solution. Although the research team designed this study as double blind, participants possibly could have communicated among themselves as to whether they swallowed expectorated after rinsing. Therefore, the study may be considered as examiner-blinded only.

Study power and statistical methods

Using the number of subjects in each group ($n = 10$) and an average standard deviation of 20 U for Periotron 8000® GCF measurements (see results, Table 1), a post-study power calculation indicated that this study had approximately 80% power to detect a mean difference of 27 GCF units between two groups ($p \leqslant 0.05$; two sided t-test).

The efficacy differences between groups at each visit were tested with an ANCOVA model, with the drug as the fixed effect, and baseline values as the covariate. The two SBG groups were compared pair-wise against the control group for the primary variable of GCF using the Dunnett test. Because the GI and Pl.I scores were not normally distributed at baseline or at the end of study, statistically significant different differences in the median GI and PLI scores between groups at each time interval (95% confidence interval) were estimated using the Kruskal–Wallace non-parametric method. The change from baseline to study termination (24 days) for these indices was normally

Table 1. Rates of gingival crevicular fluid in experimental area in Periotron units (mean ± SD)

Experimental groups	SBG rinse and swallow	SBG rinse and expectorate	Control rinse	*p*-value
Visit 1 (day 0)	32.0 ± 27.1‡	26.6 ± 12.6	23.4 ± 18.3	0.64*
Visit 2 (day 7)	46.7 ± 27.4‡	21.7 ± 12.2	23.8 ± 26.4	0.04*
Visit 3 (day 14)	20.8 ± 7.1	20.8 ± 13.2	18.0 ± 14.0	0.84*
Visit 4 (day 17)	26.8 ± 22.3	25.4 ± 16.6	32.7 ± 13.8	0.63*
Visit 5 (day 21)	24.8 ± 9.8	22.5 ± 10.8	35.4 ± 15.4	0.06*
Visit 6 (day 24)	23.4 ± 17.2	20.5 ± 10.1	34.4 ± 17.4	0.12*
Visit 1–6	$p<0.001$†	$p<0.001$†	$p<0.001$†	

*ANCOVA, $n = 30$.
†Paired *t*-test, $n = 10$.
‡$p<0.002$.
SBG, soluble β-1,3/1,6-glucan.

Table 2. Plaque index and gingival index from baseline to end of study

	Mean ± SD within group *p*-value			
Experimental groups	SBG rinse and swallow	SBG rinse and expectorate	control rinse	*p*-value
Gingival index				
Visit 1	0.24 ± 0.36	0.10 ± 0.13	0 ± 0	0.0676*
Visit 6	1.38 ± 0.27	1.45 ± 0.44	1.43 ± 0.50	0.9629*
Difference	1.16 ± 0.29	1.35 ± 0.44	1.43 ± 0.50	
	$p<0.0001$	$p<0.0001$	$p<0.0001$	
Plaque index				
Visit	0.11 ± 0.25	0 ± 0	0.03 ± 0.08	0.2535*
Visit	1.81 ± 0.29	1.68 ± 0.33	1.85 ± 0.17	0.3599*
Difference	1.70 ± 0.34	1.68 ± 0.33	1.83 ± 0.21	
Visit 1–6	$p<0.0001$†	$p<0.0001$†	$p<0.0001$†	

SBG, soluble β-1,3/1,6-glucan.
*Kruskal–Wallace test, $n = 30$.
†Paired *t*-test, $n = 10$.

distributed, and hence change within treatments over time was tested using paired *t*-tests.

Results

GCF in the test areas of the control-rinse group increased over time from baseline to 24 days ($p \leqslant 0.001$; Table 1) and GCF in the test areas of the two SBG-rinse groups decreased from days 0 to 24 ($p \leqslant 0.001$; Table 1). Subjects who rinsed and swallowed the SBG mouthrinse displayed significantly higher GCF secretion at day 7 compared with the two other groups ($p = 0.04$; Table 1). Except for this, there was no difference between the groups at any time interval.

As expected, the GI and the PLI increased in all three groups over time ($p \leqslant 0.001$; Table 2). Although the PLI and GI tended to be lower in subjects using the SBG rinses compared with those using the control rinse, there were no statistically significant differences between the test and control rinses at any time interval.

No adverse events related to the study medication were recorded and none of the blood or urine analysis values indicated any negative physiological effects of the SBG rinse (data not shown).

Discussion

The primary objective of the present randomized, single-blind intervention study was to test whether an immune modulating β-1,3/1,6-glucan could affect the development of experimental gingivitis in healthy adult humans. The experimental product used in the present study was a pharmaceutical grade SBG, known to activate immune cells in mucous tissues (Raa et al. 2000), enhance infection defense and wound healing mechanisms by reacting with specific receptors on white blood cells localized in mucosal tissues (Williams et al. 2003, Henson 2005, Luhm et al. 2006) and to counteract sepsis induced by bacterial endotoxins (Sandvik et al. 2007).

Gingivitis was experimentally induced in the study subjects by preventing cleaning of all teeth in the maxillary right quadrant of the mouth, using an individually adapted dental guard for each study participant. Gingivitis was clinically evident at day 14 in the control group and then followed a pattern, in accordance with previous experience with the experimental gingivitis model (Løe et al. 1965, Santi & Bral 1998, Majola et al. 2000, Brunet et al. 2001, Miranda et al. 2001). In the SBG swallow group there was a significantly stronger inflammatory response, as assessed by GCF, at day 7 compared with both the SBG expectoration- and the control-rinse group. This difference was not observed at any other time interval. The difference between the control and SBG swallow group at day 7 may indicate that the test substance increases the clinically invisible inflammatory parameters (GCF), in the start of the study when the plaque challenge may be ''acceptable'' to the organism. However, the SBG expectoration group showed a significant reduction from baseline to day 7 that was maintained throughout the study, indicating a suppressive reaction to the plaque challenge. This may be explained by differences in the local and systemic effects of SBG.

The results of the present study indicate that swallowing SBG may enhance low-grade gingival inflammation that is normal in adults during the first week of experimental gingivitis. This response is known from previous studies on other infections (Seljelid 1990, Robertsen et al. 1994, Bohn & BeMiller 1995, Engstad & Robertsen 1995, Franz et al. 1995, Misaki & Kakuta 1997, Williams 1997). This initial response, leading to enhanced GCF secretion in the swallow group, may therefore be the manifestation of an enhanced mucosal immune reaction that strengthens the infection defense mechanisms of the gingival tissues.

No adverse effects were observed as a result of SBG. While no data were collected about the use of contraceptives, it is possible that an extensive use of contraceptives by the females may have masked the observation of

menstrual effects on gingival inflammation or GCF.

In this study we have tested a proven immunostimulating molecule using a 24-day experimental gingivitis model and observed a reduced inflammatory response as measured by GCF flow rate. Moreover, a significantly increased inflammatory response, as measured by GCF flow rate, was also found after 7 days of swallowing the SBG as compared with the control. As stated initially, chronic gingivitis is generally defined as a reversible, non-specific bacterial infection in the gingival soft tissues (Page & Schroeder 1982, Schätzle et al. 2003). It has also been suggested that sites that bleed repeatedly on probing, i.e. showing signs of gingivitis, have a significantly higher risk for future attachment loss than sites without bleeding (Lang et al. 1986). This observation has lead to suggestions that gingivitis is a pre-requisite for the initiation of periodontitis (periodontal disease) (Schätzle et al. 2003). However, it is also clear that traces of gingivitis (or gingival inflammation) are normal in humans, including those deemed clinically healthy (Claffy 2003). In experimental gingivitis, despite an initial increase at day 7, SBG may be able to reduce the inflammatory response as measured by GCF, to a level that is lower than baseline values. Further studies should address the possible mechanisms of SBG in the inflammatory process and its possible role in affecting the development of periodontal disease in man.

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Clinical Relevance

Scientific rationale for the study: β-1,3/1,6-glucan is known to activate immune cells in mucosal tissues and thereby enhance infection defense and down-regulate inflammation caused by infectious microorganisms. This investigation focused on the clinical effect of a newly developed SBG on experimentally induced gingivitis in man.

Principal findings: GI and Pl.I increased in both the experimental groups and control group throughout the study, with no significant differences between the groups. However, secretion of GCF decreased significantly from the first to the last visit in the two test groups while GCF increased significantly during the same period in the control group. Interestingly, the group who rinsed and then swallowed the SBG showed an initial increase in GCF at day 7. Apart from this, no differences in other measured parameters (GCF) were found between the test and control groups at any time interval.

Practical implications: The results of the study indicate that SBG may interfere with the inflammatory response in experimental gingivitis as measured by GCF. Further studies should address the possible mechanisms of SBG in the inflammatory process and its possible role in affecting the development of periodontal disease in man.