The systemic effect of a food additive on dental plaque and calculus

Sune Wikner1, Christina Timander2, Jan Bergström3
1. SweDenCare AB, Umeå, Sweden
2. Blommensbergsvägen 165, Hägersten, Sweden
3. Institute of Odontology, Karolinska Institutet, Huddinge, Sweden

Short title: A food additive and dental deposits

Abstract
A food additive containing the brown algae Ascophyllum nodosum SW1313 was used in an eight-week clinical trial with the purpose to explore its possible effect on reducing supragingival plaque and calculus. 105 subjects were randomly assigned to one of three groups, each containing 35 subjects at baseline. Two groups received tablets of either a low (125 mg) or a higher (250 mg) concentration of the algae. A third group (control) received placebo tablets. Significant reductions in plaque levels compared to control were observed after eight weeks in both the low concentration (49 %) and the high concentration (66 %) algae groups ($P = 0.002$). Significant reductions in supragingival calculus levels were observed after four weeks ($P = 0.037$) improving after eight weeks ($P < 0.001$). The effect appeared to be dose-dependent. It is concluded that a food additive containing brown algae reduces the amounts of supragingival plaque and calculus in humans.
Key words : Dental calculus, dental plaque, food additive

Corresponding author: Sune Wikner, Tråggränd 6, 906 26 Umeå, Sweden

Introduction
Several attempts have been made to prevent the formation of dental plaque and calculus by topically applied chemotherapeutic agents, e g., by incorporating antiseptics in dentifrices and mouthwash. There is currently, however, limited knowledge about the possibility of interfering with plaque and calculus formation using agents via the systemic route.
One of the authors of the present study observed that a patient who normally formed a lot of calculus had none after he had changed his diet quite significantly. He had started eating a salad containing particular fresh seaweed: Ascophyllum nodosum (AN). Pilot tests with tablets made from dried AN confirmed in other patients that calculus appeared to become reduced. Since the tablets were swallowed whole - neither chewed nor sucked - the observations, interestingly, suggested that the calculus reduction may have been associated with the use of AN tablets.
AN is a brown seaweed confined to the North Atlantic basin. AN is widely used in the production of alginic acid and caregeenan, polysaccharides commonly used as food additives in dairy products. It is also used as an additive in cattle feed.
AN contains a large number of nutrients (Table 1) such as alginic acid, phenol, fucoidan, mannitol, laminaran, and sulphated fucoidan in high concentrations. Considering recommended daily allowances (RDA), high concentrations of vitamin D and iodine also are present. AN also contains several minerals and trace elements (1) e. g., magnesium, manganese, zinc, cobalt, chromium, and selenium that are vital to enzymes or hormones.

Dental plaque and calculus are common in adults (2). Besides mechanical elimination of plaque and calculus several chemical compounds have been used for this purpose e. g., triclosan, chlorhexidine, sodium lauryl sulphate and essential oils (3-4). Polyphenols such as tannins abundant in most herbs, bushes and trees and also in tea, coffee, cacao and wine have antiplaque activity in vitro and in vivo (5-6). Also cranberry has been suggested for plaque inhibition due to ability to interfere with aggregation and adhesion of S mutans (9).
Topically applied compounds, e. g., zinc (8), silica (9), and pyrophosphate as well as structural analogs of pyrophosphate, e. g., bisphosphonates have also been suggested for topical oral application to prevent calculus formation (10, 11).
Several studies have reported an influence on the calculus formation rate by altering the composition and consistency of the diet. However there is little evidence of a systemic influence in those studies. In addition, a problem in such studies is to distinguish a systemic from a topical effect.
No one has yet provided a theory of calculus formation that satisfactorily explains all the observed variations in calculus formation in humans, e. g. explained why calculus is present in some persons with an excellent oral hygiene and absent in others with a poor oral hygiene.
The purpose of the present clinical trial was to investigate the effects of AN on plaque and calculus in persons with existing supra-gingival calculus.

Material and methods

150 subjects were recruited after an advertisement in a local paper in Stockholm, Sweden. After a primary screening, 105 subjects meeting the inclusion criterion of visible deposits of supra-gingival calculus were admitted to enter the study. Persons with advanced periodontal disease were excluded. Participants were randomly assigned to one of three study groups, each containing 35 subjects at baseline.
The clinical examination comprised assessments of plaque and calculus on three tooth surfaces per person. The assessment of plaque was based on the lingual surface of 26, and the buccal surfaces of 11 and 31. Supra-gingival calculus was assessed on the opposite surfaces, respectively, of the same teeth. The extension of plaque as well as calculus was scored using the Greene and Vermillion Oral Health Index Short Form (12). In this system score 0 indicates absence of plaque or calculus, score 1 that one third or less of the gingival part of the tooth surface is covered, score 2 that two thirds are covered, and score 3 that more than two thirds are covered. Plaque and calculus mean scores per person were found from summarizing across surfaces and dividing by three. All individuals contributed the same number of scores. A preselected tooth, when missing, was replaced by 27, 41, and 21, respectively.
The food additive selected was the brown algae Ascophyllum nodosum. Tablets (ProDen PlaqueOff®) containing dried and pulverised AN were swallowed and in order to minimise any local oral effect during intake the tablets were wax-coated.
Tablets of two different concentrations of AN were tested: 125mg and 250 mg. Two experimental groups received tablets of either low (LA) or high (HA) concentration and a third group (control) received placebo tablets. Participants were instructed to take two tablets a day for four weeks when a second examination was made. Following the four-week examination, participants were instructed to take three tablets a day for a further four weeks. Eight weeks after baseline a final examination was conducted. Subsequent to the final examination, any remaining plaque and calculus were removed and the participants answered a questionnaire about adverse effects.
The participants were instructed that the tablets should be swallowed whole. The tablets were delivered to the participants in containers labelled A, B or C. Participants were asked to maintain their normal oral hygiene regimes during the course of the trial. Neither participant nor examiner was aware of the contents of tablets.

Statistics

The baseline distributions of individuals with reference to plaque and calculus were Gaussian shaped. ANOVA, repeated measures design, was used for testing the significance of differences between groups over time. The Scheffé test was applied for post hoc multiple comparisons testing. Statistical significance was accepted at $P < 0.05$.

Results

14 persons dropped out during the course of the trial, leaving a total of 29 participants in the Algae high group and 30 respectively in the others. 47 were women and 42 men. The mean age was 45 years (range 23-68 years) and there were no significant differences between groups. Adverse events were rare. One person in a test group complained about abdominal pain.

The mean levels of plaque and calculus at baseline and during the course of the trial are presented in Tables 2 and 3, respectively. There were no statistically significant differences between groups at baseline. After four weeks, slight reductions of plaque were observed in all groups. There were, however, no significant differences between groups. Further reductions were observed at eight weeks in both the low concentration alga (LA) and the high concentration alga (HA) groups but not in the placebo group (Table 2). The reductions compared to baseline were 49 % and 66 % in LA and HA groups, respectively, compared to just 20% in the placebo group. The differences between groups were statistically significant ($P = 0.002$). Post hoc comparisons testing revealed a significant difference between HA and placebo groups (Scheffé $P = 0.002$) but not between LA and placebo groups or LA and HA groups.

The changes in plaque levels over time are illustrated in Figure 1. Repeated measures ANOVA disclosed a significant plaque reduction effect over time associated with substance use. Although differences between groups regarding change were small during the first 4 week period, there were significant differences between LA and placebo groups and between HA and placebo groups during the second 4 week period ($P = 0.038$ and $P = 0.015$, respectively). For the total duration of the trial, the differences in plaque reduction between LA and placebo groups as well as between HA and placebo groups were statistically significant ($P = 0.015$ and $P = 0.001$, respectively).

There were no statistically significant differences with reference to calculus extension between groups at baseline. After four weeks, however, a statistically significant reduction associated with the test substance was observed ($P = 0.037$). Further reductions were observed after eight weeks in LA and HA groups but not in the placebo group. The differences between groups were statistically significant ($P < 0.001$). Post hoc comparisons testing suggested significant differences between HA and placebo groups as well as between LA and placebo groups (Scheffé $P < 0.001$ and $P = 0.004$, respectively). The difference between LA and HA groups was almost significant ($P = 0.080$). The changes in calculus levels over time are illustrated in Figure 2. Similar to plaque changes, a significant calculus reduction effect over time associated with substance use was observed. The differences in calculus reduction between groups during the first 4 weeks were not significant. During the second 4 week period, however, a significant difference was seen between HA and placebo groups ($p = 0.029$) and a trend towards a difference between LA and placebo groups ($p = 0.078$). For the total eight-week period the calculus reductions in both LA and HA groups were statistically significant compared to placebo ($P = 0.015$ and $P = 0.001$, respectively). The results remained as baseline plaque levels were introduced as a co-variable in the analyses.

Discussion

This is the first study to demonstrate that a food supplement containing the brown algae Ascophyllum nodosum taken daily, significantly reduces the amount of established supragingival plaque and calculus in humans. The higher concentration of the algae had a greater effect than the lower one, suggesting a dose-dependent response. This is further substantiated by the observation that a greater reduction was seen on s three tablets daily regime during the second phase of the trial than on two tablets daily regime during the first phase.

The reduction of plaque in the placebo group at the four-week examination reflected an improvement in oral hygiene during the first part of the study, making difficult any firm conclusion about the true reducing effect of the active agent during the first four weeks. However, at the eight-week examination the plaque level of the placebo group remained unchanged whereas the levels observed in the verume groups continued to decrease. The mechanisms that may account for the reductions observed remain unknown. Given that the tablets were wax-coated and swallowed, it would appear that the effect resulted from systemic absorption of algal components that eventually changed the composition and property of saliva.

Among the components of AN, phenols, iodine and sulphate have previously been shown to affect plaque accumulation when used topically (5-7, 13). The sulphate containing fucoidan in AN may thus be responsible for the plaque reduction observed. This is in line with the results of previous studies that have demonstrated that funoran, like fucoidan a sulphated polysaccharide, has a strong desorbtion activity against mutans streptococci pre-adsorbed to saliva- coated hydroxyl apatite. Funoran reduces the colonisation of S. cricetus in animals and also the caries scores compared to a control group (14). However, the active agent in those studies was added to drinking water which precludes the possibility to judge if the effect was systemic or local. Added to chewing gum funoran reduced plaque formation in man (15). It is assumed that the sulphate groups of funoran interfere with bacterial adhesion to proteins of the pellicle.

About 35 per cent of AN dry weight consist of highly acid sulphate esters and the absorption and mobilisation of these compounds may contribute to an increased sulfation of acid salivary components such as glycosaminoglycans (GAGs). The acidic nature of these components exhibits calcium-binding properties, which in turn would reduce the concentration of calcium available for precipitation (16).

Hydroxyapatite is resistant to demineralisation but may dissolve when exposed to acids produced by oral bacteria. The critical pH for dissolution of hydroxyapatite in dental enamel is 5,5 in persons with high salivary calcium and phosphate concentrations (17). It is reasonable that calculus dissolves at a higher pH than does hydroxyapatite due to its content of soluble calcium phosphates and carbonates.

Another possible explanation to the dissolution of calculus is acids from microorganisms in plaque. The bacterial composition of dental plaque should be the same as in plaque covering dental calculus. Thus after consumption of fermentable carbohydrates a pH drop should occur on the calculus surface having the same magnitude as on the enamel surface. Consequently one may expect that a pH drop in plaque covering calculus will dissolve calculus and particularly if the calculus contain calcium precipitations that dissolve at a higher pH than hydroxyapatite in dental enamel.

In conclusion, the results of the present study suggest that daily consumption of AN containing tablets reduces dental plaque and calculus in a dose dependent manner. It seems that the effect is related to systemic absorption and distribution to saliva of certain components of AN. Future studies will be needed to further explore the mechanisms involved.

References

1. Chapman VJ, Chapman DJ. In: Seaweeds and their uses. 3rd edition. London: Chapman and Hall, 1980; 30-61.

2. Albanda JM, Kingman A. Gingival recession, gingival bleeding and dental calculus in adults 30 years of age and older in the United States 1988- 1994. J Periodontol 1999; 70 : 30-43.

3. Waaler SM, Rölla G, Skjorland KK, Ögaard B. Effects of oral rinsing with triclosan and sodium lauryl sulfate on dental plaque formation: a pilot study. Scand J Dent Res 1993 ; 101: 192-5.

4. Overholser CD, Meiller TF, De Paola LG, Minah GE, Niegaus C. Comparative effects of two chemotherapeutic mouthrinses on the development of plaque and gingivitis. J Clin Periodontol 1990; 17: 575-9.

5. Matsomoto M. et al. Inhibitory effects of cacao bean husk extract on plaque formation in vitro and in vivo. Eur J Oral Sci 2004; 112: 249-52.

6. Bhattacharya S, Virani S, Zavro M, Haas G. Inhibition of Streptococcus Mutans and other oral streptococci by hop (Humulus Lupulus) constituents. Economic Botany 200; 57:604-18.

7. Weiss EI et al. Inhibiting interspecies coaggregation of plaque bacteria with cranberry juice constituent. J Am Dent Assoc 1998; 129: 1719- 23.

8. Ingram GS, Horay CP, Stead WJ: Interaction of Zinc with dental mineral. Caries Res 1992; 26: 248-53.

9. Sowinski JA et al. A clinical study to assess the anticalculus efficacy of a new dentifrice containing a special grade of silica : a clinical trial on adults. J Clin Dent 2002; 13: 65-8.

10. Vogel JJ, Amdur BH. Inorganic pyrophosphate in parotid saliva and ist relation to calculus formation. Archs Oral Biol 1967; 12: 159- 63.

11. Fleish H. Biphosphonates: Mechanisms of action. Endocr rev 1998; 19: 80- 100.

12. Greene JC, Vermillion JR. The oral hygiene index: a method for classifying oral hygiene status. J Am Dent Assoc 1960; 61: 172- 9.

13. Rosling et al. The use of PVP- iodine as an adjunct to non- surgical treatment of chronical periodontitis. J Clin Periodontol 2001; 28: 1023- 31.

14. Saeki Y, Kato T, Takazoe I, Okuda K. Inhibitory effects of funoran on the adherence and colonization of mutans streptococci. Caries Res 1996; 30: 119-25.

15. Sato S et al. The inhibitory effect of funoran and eucalyptus extract- containing chewing gum on plaque formation.J Oral Sci 1998; 40: 115- 7.

16. Embery G, Rölla G, Stanbury JB. Interaction of acid glucosaminoglycans (mucopolysaccharides) with hydroxyapatite. Scand J Dent Res 1979; 87: 318-24.

17. Ericsson Y. Enamel- apatite solubility. Investigations into the calcium phosphate equilibrium between enamel and saliva and its relation to dental caries. Acta Odont Scand 1949; 8 (Suppl 3): 1- 139.

Table 1. Average composition of the brown algae Ascophyllum Nodosum.

Major components	**%**
N- free extractives	45-60
Alginic acid	20-26
Phenol	5-15
Mannitol	5- 8
Laminaran	2- 5
Fucoidan	10-15
Crude fibre	8
Crude protein	5-10
Lipid	2

Minor minerals	**mg/kg**
P	1500
I	500-1200
Fe	150-1000
Zn	50-200
B	40-100
Mn	10-50
Ba	15-50
Co	1-10
Cu	1-10
Se	4
Ni	2-5

Major minerals	**mg/kg**
S	35000
Cl	37000
Na	35000
K	25000
Ca	20000
Mg	7000

Vitamins	**mg/kg**
C	500-200
E	150-300
K	10

Table 2. Variation of plaque over time. Mean and standard deviation

	Baseline		4 weeks		8 weeks	
	Mean	SD	Mean	SD	Mean	SD
Algae high	4.72	1.71	3.17	1.61	1.62	1.41
Algae low	5.01	1.70	3.90	2.05	2.56	2.02
Placebo	4.26	1.54	3.40	1.71	3.40	2.07
	F = 1.6, P > 0.05		F = 1.2, P > 0.05		F = 6.8, P = 0.002	

Table 3. Variation of supragingival calculus over time. Mean and standard deviation

	Baseline		4 weeks		8 weeks	
	Mean	SD	Mean	SD	Mean	SD
Algae high	3.07	1.16	2.31	1.01	1.65	1.04
Algae low	3.33	1.69	2.89	1.51	2.40	1.53
Placebo	3.43	1.26	3.16	1.42	3.50	1.31
	F = 0.6, P > 0.05		F = 3.4, P > 0.037		F = 16.2, P = 0.001	

Fig 1

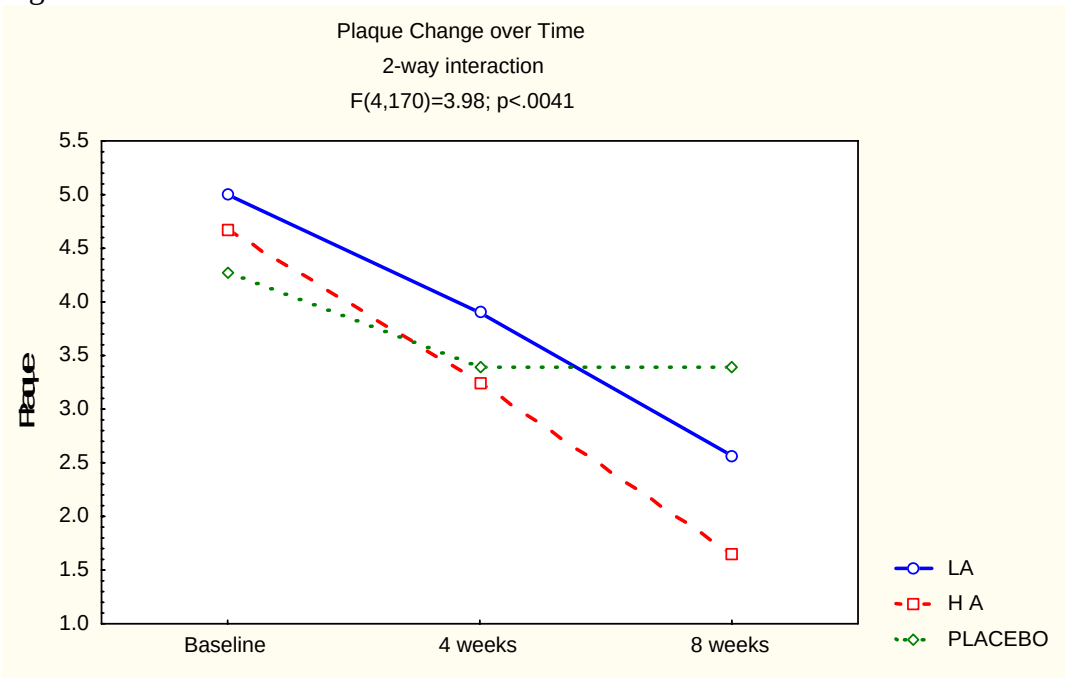
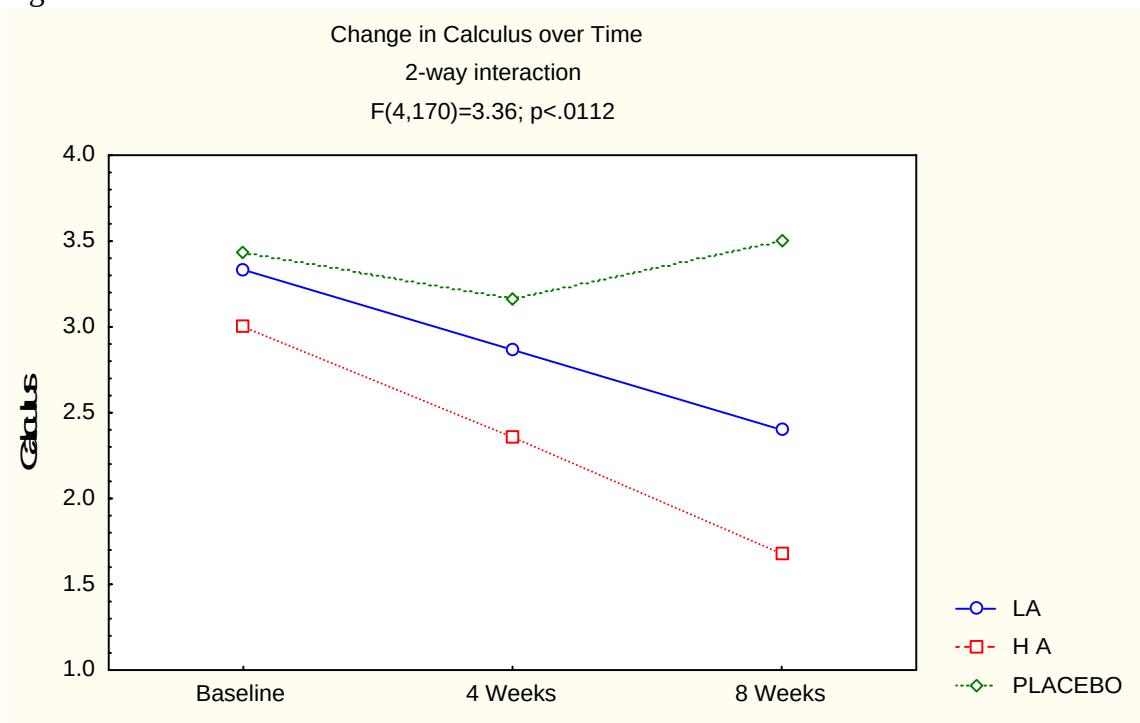

Plaque Change over Time
2-way interaction
F(4,170)=3.98; p<.0041
Plaque
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Baseline
4 weeks
8 weeks
LA
H A
PLACEBO

Fig 2


Change in Calculus over Time
2-way interaction
F(4,170)=3.36; p<.0112
Calculus
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Baseline
4 Weeks
8 Weeks
LA
H A
PLACEBO