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Anticalculus effect of a triclosan mouthwash containing phytate: a double-blind, randomized, three-period crossover trial

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Background and Objective: Dental calculus occurs as a consequence of supersaturation of saliva with respect to calcium phosphates. This mineralization of dental plaque can be delayed by the presence of crystallization inhibitors, such as pyrophosphate or bisphosphonates. Phytate inhibits brushite and hydroxyapatite crystallization and has the potential to prevent dental calculi formation. The aim of the present study was to examine the effects of phytate and zinc, administered in a mouthwash solution, to prevent the formation of dental calculus.

Material and Methods: Healthy dental plaque-forming volunteers ($n = 25$) took part in a randomized, double-blind, three-period crossover clinical study to assess the efficacy of a phytate-containing mouthwash in relation to control and placebo effects. Subjects rinsed their mouths for 1 min, twice each day, with 20 mL of the test solution, without ingestion. Mouthwash efficacy was assessed through quantification of the amounts of calcium, phosphorus and magnesium present in the residues obtained by dental cleaning, performed by a single trained examiner.

Results: A good correlation was found among total calcium, magnesium and phosphorus in calcified dental plaque residues, indicating that any of these variables is adequate for evaluating the reduction of plaque crystallization as calcium phosphate. A statistically significant decrease in total calcium, magnesium and phosphorus was found in the phytate-treatment period compared with control and placebo periods, demonstrating the efficacy of the proposed treatment in reducing dental calculus formation.

Conclusion: The high efficacy of phytate in reducing dental calculus formation suggests that this substance may be an effective treatment for preventing the development of calculus deposits.

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Dental calculus is composed of inorganic components and an organic matrix. Different calcium phosphates (mainly hydroxyapatite and brushite) make up the mineral components of dental calculus. Salivary proteins adsorb onto the tooth surface to form a continuous film to which diverse oral microorganisms adhere. Following initial bacterial adherence to the tooth surface, complex communities of microorganisms develop and form a

biofilm, which has significant resistance to antimicrobial agents (1). Supersaturation of saliva with respect to calcium phosphates is the thermodynamic driving force for plaque mineralization. The mechanism involved is similar to that responsible for the development of concretions around urinary catheters (2) and in the formation of some types of renal calculi (3). The presence of crystallization inhibitors can prevent or delay formation of the mineral phase.

Pyrophosphate was the first and one of the most common ingredients used for dental calculus control (4–7). Clinical studies have shown that pyrophosphate formulations can reduce the development of calculus by 20–40% (8–11). Crystallization inhibitors bind to crystal nuclei or crystal faces and disrupt crystal development. The adsorption of such compounds to crystal faces can also inhibit crystal dissolution. In fact, since the 1930s it has been known that trace amounts of certain molecules, including polyphosphates, can act as water softeners through inhibiting the crystallization of calcium salts, such as calcium carbonate. However, the use of such compounds as regulators of calcification under physiological conditions was not explored until the 1960s when it was shown that pyrophosphate, a naturally occurring polyphosphate, was present in serum, urine and other biological fluids, and could prevent calcification by binding to hydroxyapatite (12,13). While pyrophosphate is safe and effective, there are some limitations in its oral use. Thus, once adsorbed onto the tooth or calculus nucleation surface sites, pyrophosphate can be de-activated, mainly by hydrolysis, as a result of either enzymatic (phosphatase) activity or aqueous degradation. The search for stable synthetic analogues of pyrophosphate, which might also have crystallization-inhibitory properties combined with resistance to hydrolysis, led to the formation of bisphosphonates. Etidronate has been demonstrated to prevent dental calculus (14,15).

Phytate (myo-inositol hexakisphosphate) is abundant in plant seeds and is also found at low levels in all mammalian organs, tissues and fluids (16,17). Phytate has a pronounced inhibitory effect on brushite and hydroxyapatite crystallization (18) and, along with other polyphosphates, has been shown to inhibit calcium salt crystallization in urine and soft tissues (19,20). The inhibitory effects of phytate on calcium oxalate crystallization are increased by the presence of some metallic cations, including zinc (21).

The present study examined the abilities of phytate and zinc, administered in a mouthwash solution, to prevent the formation of dental calculus.

Material and methods

Participants

The study population consisted of healthy employed adult volunteers who provided informed consent, met the entrance criteria and did not present the exclusion criteria outlined in Table 1. Prior to enrollment in the study, dental calculus was removed from the subjects' teeth by dental cleaning and used to determine total calcium, following the procedure indicated in the 'Outcomes' section.

Personal and clinical data were collected by the Dental Clinic Periodent (Palma de Mallorca, Spain).

This research study was approved by the Balearic Research Ethics Board.

Interventions

In a randomized, double-blind, three-period crossover clinical study the efficacy of a phytate-containing mouthwash was assessed. Using this study design, each participant under went three treatments, the results of which were compared within individual patients and it was possible to ensure that there was no effect of the treatment order. The study was conducted in three phases over a 9-wk period and involved a control period (no mouthwash treatment for 3 wk), a placebo period (mouthwash with 0.001% zinc for 3 wk) and a phytate-treatment period (mouthwash with 0.001% zinc and 0.1% phytate for 3 wk).

After each period, complete extraction of all calcified dental plaque was performed by a single trained examiner, to avoid differences between investigators. All residues of dental plaque were removed through dental cleaning, using a bone collector aspirator with an attached disposable filter (Mozo-Grau, Valladolid, Spain) to aspire and collect all calculus fragments. The filters with the collected samples were placed in a sterile plastic container until analysis in the laboratory. A wash-out period was considered unnecessary.

Subjects rinsed their mouths for 1 min twice each day with 20 mL of the appropriate solution, without ingestion. The compositions of the mouthwash solutions are shown in Table 2.

Patients continued with normal dental hygiene practice using the provided toothpaste, without additives with crystallization inhibitory properties, twice a day (after breakfast and dinner).

Objectives

The hypothesis for the study was that phytate and zinc reduce the degree of hydroxyapatite formation in dental plaque. The objective of the study was to assess whether a phytate-treatment period of 3 wk using a mouthwash containing phytate and zinc could

Table 1. Criteria for subject acceptance into the study

Entrance criteria	Exclusion criteria
Age between 18 and 65 years	Known hypersensitivity to any components of the mouthrinse solutions
Previously presented calcified dental plaque with calculus elimination	Oral active infection
Agree to participate in the study and to provide informed consent	Need of other treatments intramouth
Have total calcium in dental plaque greater than 0.5 mg	Severe systemic diseases
	Risk of pregnancy
	Drug dependence

Table 2. Composition of the mouthwash solutions used in the study, expressed as percentage by weight

Compound	%
Placebo period	
Ethanol	3
Sorbitol	4
Lutrol F127	1
Zinc chloride	0.00209
Menthol	0.0425
Triclosan	0.012
Sodium saccharin	0.01
Sodium benzoate	0.1
Colorant (CI 47005 and CI 42051)	0.015
Phytate-treatment period	
Ethanol	3
Sorbitol	4
Lutrol F127	1
Zinc chloride	0.00209
Menthol	0.0425
Triclosan	0.012
Sodium saccharin	0.01
Sodium benzoate	0.1
Colorant (CI 47005 and CI 42051)	0.015
Potassium phytate	0.142

significantly reduce the calcification (as hydroxyapatite) of dental plaque in relation to control and placebo periods.

Outcomes

Phytate-treatment efficacy was assessed by evaluating the amount of calcium (primary outcome measure), phosphorus and magnesium (secondary outcome measures) deposited in dental plaque during each treatment period (3 wk).

Residues were then dissolved with 1 M HCl, and the concentrations of calcium, phosphorus and magnesium were determined using inductively coupled plasma atomic emission spectrometry (Optima 5300DV spectrometer; Perkin-Elmer S. L., Madrid, Spain).

A directed anamnesis was applied to each volunteer to study tolerability and possible side effects.

Sample size

Twenty participants were required to detect as significant (at the two-sided 5% level) a reduction of at least 30% in total calcium in the active treatments vs. control period, with an expected amount of calcium of 1.00 mg (standard deviation: 0.45 mg), based on 80% power. To compensate for nonevaluable patients, it was planned to enrol 25 patients in the study.

Randomization

Participants had an equal probability of assignment to the treatment sequence. The randomization table was developed using a computer random number generator to select random permuted blocks. The block lengths were six, which were known to the investigators, although they did not know the mouthwash they were administering.

Allocation

A single trained examiner received a code table to assign the blinded treatment, identically packed with a coded label, to each volunteer.

Blinding

With the exception of the control period, all subjects and investigators were blinded to subject period assignment. The treatment assignments were unblinded after statistical analysis.

Statistics

Continuous variables were described in terms of the mean, standard deviation, median and range, proportions and 95% confidence intervals. The treatment was tested, allowing for possible period effect, using ordinary least-squares analysis (22). Data were tested for normality using Shapiro–Wilk's test. As data were not normally distributed, the variables were log transformed and the estimate of the treatment effect was antilogged and expressed as a ratio. These calculations were made using the SAS statistical package, version 8.02 (SAS Institute Inc., Cary, NC, USA).

Results

A total of 31 healthy adult subjects were enrolled in the study from April 2006 to February 2007. Of these, five did not meet the entrance criteria and one was lost to follow-up. The baseline data for the 25 included subjects are presented in Table 3.

Table 3. Demographics of the selected volunteers

Characteristic	Value (n = 25)
Mean age ± SE	35.2 ± 3.4
Gender, n (%)	
Male	13 (52)
Female	12 (48)
Mean body mass index ± SE, kg/m^2	24.0 ± 0.3

Table 4 and Fig. 1 summarize the results from the experimental phase. Following the phytate-treatment period, a reduction in total calcium in the collected residues of developed dental calculus was observed in all subjects compared with those in the control period, and a reduction was observed in 24/25 subjects compared with the placebo period.

With respect to phosphorus, following the phytate-treatment period 23/25 subjects showed a reduction in total phosphorus in collected residues of developed dental calculus compared with both the control and placebo periods.

With respect to magnesium, following the phytate-treatment period 23/25 subjects showed a reduction in total magnesium in collected residues of developed dental calculus compared with the control period, and 19/25 showed a reduction compared with the placebo period.

Figure 1 summarizes the effects of phytate treatment on total calcium, phosphorus and magnesium in dental calculus compared with the control and placebo effects. There was a statistically significant decrease in these three elements in the phytate-treatment period compared with the control period, demonstrating clear efficacy of the phytate treatment in reducing dental calculus.

Table 5 shows the calcium, phosphorus and magnesium mean values, with 95% confidence intervals, median and 25 and 75 percentiles, after the different phases of the study.

Table 4. Calcium (Ca), magnesium (Mg) and phosphorus (P) content of collected residues of plaque that developed in subjects during each of the three treatment periods in the study

	Control period			Placebo period			Phytate-treated period		
Volunteer no.	Ca (μg)	P (μg)	Mg (μg)	Ca (μg)	P (μg)	Mg (μg)	Ca (μg)	P (μg)	Mg (μg)
1	235	152	3.4	509	1130	6.8	222	120	11.7
2	154	75	5.3	243	344	7.2	62	42	1.5
3	547	876	10.0	940	1269	13.9	54	144	3.9
4	110	44	4.0	78	45	2.6	30	13	3.0
5	22	5	2.2	83	16	2.4	16	9	1.0
6	539	377	17.7	326	146	19.1	101	26	3.3
7	457	296	16.3	277	172	2.4	106	73	2.9
8	1286	825	36.0	1234	821	26.0	205	122	4.2
9	951	617	19.6	41	18	2.0	103	65	3.7
10	530	408	6.0	553	396	6.0	425	328	3.5
11	300	187	5.2	380	251	7.5	103	68	1.8
12	293	190	7.7	116	60	3.9	59	32	1.0
13	113	35	3.5	158	98	3.1	73	46	2.7
14	402	249	5.8	690	456	10.9	122	51	1.0
15	66	34	2.2	24	6	0.3	11	4	0.1
16	94	53	0.7	145	10	3.4	53	27	1.0
17	1086	680	21.7	1122	602	23.4	316	217	6.5
18	1056	736	19.7	736	686	11.5	202	143	3.7
19	727	511	10.2	344	241	10.1	112	80	4.2
20	1718	1191	24.2	1927	1377	31.6	425	290	4.8
21	137	79	3.0	488	274	12.5	21	5	0.0
22	138	76	2.5	138	92	1.8	49	32	2.0
23	1963	1467	30.2	1405	1008	20.3	584	441	3.8
24	169	104	3.7	147	96	4.8	138	84	2.4
25	412	320	5.0	260	198	2.2	226	165	2.7

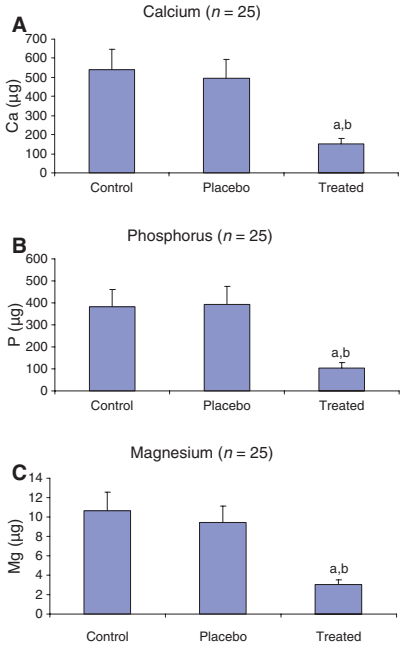

Fig. 1. Mean values for total calcium (A), phosphorus (B) and magnesium (C) in the residues of plaque that developed during the three treatment periods. Error bars represent standard error. [a]$p < 0.05$ vs. control period. [b]$p < 0.05$ vs. placebo period.

The amount of calcium and phosphorus deposited after using the mouthwash containing phytate was three times lower than after placebo. Similar reductions were observed for magnesium, whereas no statistical significance was found between the control and placebo (Table 6).

Table 6. Estimated treatment effect adjusted for period

		CI 95%	Significance
Ratio phytate/placebo			
Ca	0.33	0.24–0.45	< 0.0001
P	0.33	0.22–0.50	< 0.0001
Mg	0.40	0.28–0.56	< 0.0001
Ratio phytate/control			
Ca	0.29	0.21–0.40	< 0.0001
P	0.29	0.19–0.43	< 0.0001
Mg	0.33	0.23–0.46	< 0.0001
Ratio placebo/control			
Ca	0.89	0.65–1.21	0.43
P	0.86	0.57–1.29	0.44
Mg	0.83	0.59–1.17	0.26

The percentage of patients who accumulated 30% less calcium, phosphorus or magnesium after the phytate-treatment period (compared with either the control or the placebo period) is presented in Table 7.

A total of four subjects reported minor adverse reactions during the study. All reactions were unremarkable and were reported during the placebo and treatment periods. No subject discontinued the study because of to an adverse event.

Discussion

This clinical study showed that the presence of phytate and zinc in mouthwash solution significantly inhibited the development of dental calculus. Thus, the phytate/zinc treatment resulted in a reduction of

Table 5. Calcium (Ca), magnesium (Mg) and phosphorus (P) content of collected residues of plaque that developed in subjects during basal conditions and after 3 wk of each treatment

	Mean	SD	CI 95%		Median	P25	P75
Ca (μg)							
Basal	2376.0	1721.3	1665.5	3086.5	2120.0	580.0	3860.0
Control period	540.2	526.8	322.7	757.7	402.0	137.5	839.0
Placebo period	494.6	489.3	292.6	696.6	326.0	141.5	713.0
Phytate-treatment period	152.7	146.3	92.3	213.1	103.0	53.5	213.5
P (μg)							
Basal	1464.8	1108.8	1007.1	1922.5	1290.0	355.0	2540.0
Control period	383.5	391.5	221.9	545.1	249.0	75.5	648.5
Placebo period	392.5	421.2	218.6	566.4	241.0	76.0	644.0
Phytate-treatment period	105.1	110.4	59.5	150.7	68.0	29.5	143.5
Mg (μg)							
Basal	37.9	29.8	25.6	50.2	32.9	10.8	54.4
Control period	10.6	9.8	6.6	14.7	5.8	3.5	18.7
Placebo period	9.4	8.6	5.9	13.0	6.8	2.5	13.2
Phytate-treatment period	3.1	2.4	2.1	4.0	2.9	1.3	3.9

Table 7. Proportion of patients with 30% less calcium (Ca), phosphorus (P) and magnesium (Mg) after phytate treatment than after control or placebo treatment

				CI 95%	
		Frequency	%	Lower	Upper
Ca	Phytate treatment vs. control period	21	84.0	69.6	98.4
	Phytate treatment vs. placebo period				
P	Phytate treatment vs. control period	20	80.0	64.3	95.9
	Phytate treatment vs. placebo period				
Mg	Phytate treatment vs. control period	20	80.0	64.3	95.7
	Phytate treatment vs. placebo period	18	72.0	54.4	89.6

approximately 70% in tartar formation as a result of the calcification of dental plaque. The results of the present study suggest that phytate may play an important role in preventing the development of dental calculus.

The high Pearson's correlation coefficient values indicate a correlation among the three variables (calcium, phosphorus and magnesium). In the case of calcium vs. phosphorus the value of the coefficient ($r = 0.9845$), of almost 1, suggests that either of these variables is adequate for evaluating the reduction of plaque crystallization as calcium phosphate.

The correlation coefficients of calcium vs. magnesium and magnesium vs. phosphorus were close to 0.9, suggesting the formation of mixed calcium–magnesium salts that coprecipitated with calcium phosphate. The data indicate that they are minor salts although they correlated with the total amount of calcium and phosphorus.

The data are consistent with findings of the capacity of phytate to act as an inhibitor of hydroxyapatite and brushite crystal formation *in vitro* (18) and *in vivo* (20,23). The phytate molecule has structural similarities to pyrophosphate, the most common polyphosphate used for dental calculus control (4–7), which probably explains their common activity. However, it is interesting to compare dental calculus development with pathological calcification of soft tissues or some types of renal calculus formation. In all of these situations hydroxyapatite is the common mineral phase present, and the development of calcification requires a pre-existing organic phase as an inducer (heterogeneous nucleant), whereas further progression requires the presence of other promoter factors (high levels of calcium and/or phosphate) and/or the absence (or deficiency) of calcification inhibitors such as pyrophosphate and phytate (23,24).

The entrance criteria for this study were selected to include only patients with more than 0.5 mg of total calcium in dental plaque; thus, when extrapolating the results to the general population it is necessary to consider that trial participants presented a profile of severe dental calculi formation.

In conclusion, the high efficacy exhibited by phytate in reducing dental calculus formation suggests that this substance may be an effective treatment for preventing the development of calculus deposits.

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