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The Oral Disposition of Zinc Following the Use of an Anticalculus Toothpaste Containing 0·5% Zinc Citrate

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Abstract—Zinc is retained in the mouth after use of a toothpaste containing 0·5% zinc citrate. More than one third of the dose was found to be retained after normal brushing. Elevated zinc levels were also found in plaque. Saliva zinc levels were significantly above background for at least 2 h after brushing. In-vitro experiments demonstrated that zinc can bind to the pellicle-coated tooth surface and can subsequently desorb into saliva. Plaque can calcify to form calculus containing appreciable levels of hydroxyapatite. Zinc adsorbs to hydroxyapatite inhibiting crystal growth. Levels of zinc in plaque were found to be considerably higher than those taken up by hydroxyapatite in an in-vitro test of crystal growth inhibition indicating the potential of zinc to inhibit calculus formation.

Toothpastes containing zinc citrate trihydrate (ZCT) at 0·5% (w/w) are known to inhibit plaque growth (Saxton et al 1986) and calculus formation (Stephen et al 1987).

The antiplaque efficacy of zinc salts has been reported by several workers (Hanke 1940; Fischman et al 1973; Skjörland et al 1978) and zinc salts are known to have anticalculus properties in-vivo (Schmid et al 1974; Lobene et al 1985; Stephen et al 1987). These biological actions of zinc may be due to retention in the mouth after brushing with the dentifrice containing a zinc salt. Oral retention of zinc was reported by Afseth et al (1983a) (15% retention of a 2·5 mmol L^{-1} zinc acetate mouth-rinse) and Harrap et al (1984) (12% retention from a 30 mmol L^{-1} zinc phenol sulphonate mouthrinse). Uptake of zinc to plaque has also been described (Saxton et al 1986; Afseth et al 1983b), together with subsequent inhibition of bacterial acid production in response to a sucrose challenge (Afseth et al 1980; Oppermann & Rölla 1980; Opperman et al 1980).

Plaque can calcify to form calculus or tartar (Schroeder 1969). Schroeder & Bambauer (1966) found that hydroxyapatite and whitlockite were commonly present in mature calculus specimens. Compounds which inhibit the crystal growth of hydroxyapatite also have the potential to inhibit dental calculus formation (Briner & Francis 1973).

The antitartar effects of zinc salts are likely to be due to a combination of the antiplaque effect and an inhibitory action on tartar crystallization. Zinc is known to inhibit seeded hydroxyapatite crystal growth in-vitro (Ingram & Carter 1987) and Picozzi et al (1972) attributed this property to zinc, following a study in-vivo. Nuclei of hydroxyapatite, forming within the plaque, would be inhibited from further growth.

This report describes measurements of the intra-oral disposition of zinc in humans following toothbrushing with a dentifrice containing 0·5% (w/w) ZCT. Additionally, some in-vitro experiments are presented to measure uptake of zinc to the pellicle-covered tooth surface and to relate uptake of zinc by plaque and hydroxyapatite to the concentration required to inhibit seeded hydroxyapatite crystal growth in-vitro.

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Materials and Methods

Toothpaste

The toothpaste used was an alumina based dentifrice containing 0·5% w/w zinc citrate trihydrate (ZCT) and the anticaries agent sodium monofluorophosphate (0·8%). Also included in the formulation were anionic surfactant, humectant, flavour and other minor dentifrice excipients.

Human investigations

Ten volunteers gave their written informed consent for the study; prospective volunteers who were pregnant, on antimicrobial therapy or who had overt dental disease were excluded. Volunteers were asked not to brush their teeth during the 24 h preceding the test, and, when brushing, to leave untouched the inside of the back molars to conserve plaque for collection and zinc analysis. Plaque and saliva samples were taken before toothpaste use for measurement of background zinc levels. Participants then brushed their teeth with 1 g of the toothpaste, accurately weighed. A new toothbrush was used. Brushing was for 1 min after which the brush was collected for analysis of zinc remaining. Both the expectorate and a further 5 mL water rinse were collected and analysed for zinc content. After 5 min, plaque was sampled from the lingual aspect of the molars using a dental scaler and saliva samples were taken 5 min, 30 min, 1 and 2 h after toothbrushing. Care was taken to avoid those areas of the teeth where dental calculus had formed.

Zinc analysis

The principle (Delves 1981) of acid extraction of zinc from samples containing protein was utilized. Portions (2·0 g) of all samples except plaque were oxidized at 250 °C in 75 mL Kjeldahl flasks with 1 mL conc. sulphuric acid. After charring, the samples were allowed to cool and ca 1·0 mL of conc. nitric acid was added dropwise. The sample was further

heated to 300 °C until the mixture became clear. The oxidation was completed by the addition of 10 mL deionized water and heating to 150 °C to remove nitrous oxides and nitric acid. When only ca 1 mL of sample remained, the mixture was made up to 50 mL with deionized water and analysed for zinc content.

Hydroxyapatite was analysed for zinc content by dissolution of 50 mg in 1 mL conc. HCl. This was made to 25 mL with distilled water.

Analysis of all samples was by atomic absorption spectroscopy on the 213·9 nm line (0·7 nm slit width) using an air acetylene flame and deuterium background correction. A Perkin Elmer model 373 spectrophotometer (Beaconsfield, Buckinghamshire, UK) was calibrated with matched aqueous standards in 2% sulphuric acid of 1–3 $\mu g\ g^{-1}$ zinc. Samples were diluted into range with 2% sulphuric acid and zinc recovery in control experiments and standard solutions with or without toothpaste was greater than 95%.

Plaque samples were prepared by overnight digestion of the known wet weight of plaque in 1 mL concentrated nitric acid at room temperature. The volume was then made to 5 mL with distilled water and also analysed by atomic absorption spectroscopy.

Zinc uptake to hydroxyapatite

Portions of hydroxyapatite, 50 mg, were treated with 20 mL portions of zinc acetate solution or of an extract (10 g+30 mL water) of 0·5% zinc citrate toothpaste for 5 min. The hydroxyapatite was separated by centrifugation and washed twice by resuspension in 25 mL portions of water and recentrifugation. The solids were drained and used for determination of zinc uptake by acid washing and atomic absorption spectroscopy.

Zinc uptake to the tooth surface in-vitro

Human molar teeth were used, obtained from local dentists. The area of tooth exposed to test toothpaste was standardized. Each tooth was cut in half and covered in wax applied with a camel hair brush, with the exception of a 6 mm × 6 mm window left uncovered on the enamel surface of each tooth half.

Before manipulation, each tooth was cleaned by standing in 5% (w/v) aqueous sodium hypochlorite for approximately 2 h. A uniform proteinaceous pellicle was replaced by standing in saliva for 3 min. The saliva was always taken from the same person and stored frozen in aliquots for use as appropriate.

Studies of zinc uptake were carried out using 50% (w/v) aqueous slurries of toothpaste with ^{65}zinc chloride added (specific activity 8·65 $\mu Ci\ mg^{-1}$, New England Nuclear, Dreieich, West Germany). This specific activity was sufficiently high to allow very small amounts of zinc (0·125 μg) to be added as tracer for the zinc in the toothpaste.

Toothpaste was applied by dipping of the tooth into the stirred slurry for various times up to 15 min. Uptake of radiolabel was measured as follows. After application the tooth was washed for 45 s in water. The wax was then removed and discarded, while the tooth was washed twice for 30 s in 0·1 M HNO_3 (2 mL). The radioactivity collected in the two acid rinses was measured in a Packard Tricarb 4530 Scintillation Counter with appropriate quench correction. This was then expressed as the quantity of zinc binding to the tooth surface in the exposed window.

Desorption experiments were carried out to examine loss of zinc from the tooth surface into saliva. In these experiments after a period of 1 min for uptake to a wax covered tooth with a 6 × 6 mm window, the wax was removed, the tooth was placed in saliva and the desorbed radioactivity measured by scintillation spectroscopy. These experiments were carried out with various concentrations of unlabelled zinc in the saliva in order to illustrate any inhibitory effect on the desorption rate.

Results were corrected for the surface area of tooth used and expressed as μg uptake cm^{-2} tooth surface.

Results

These experiments demonstrate that zinc is retained in the mouth after brushing with the test dentifrice. The data in Table 1 summarize the mean fate of zinc in the ten volunteers. The zinc remaining on the brush is equivalent to 9·6% of the dose and is likely to be representative of toothpaste remaining residual in the bristles. Just over half (52%) of the dose of zinc in the 1 g dose was recovered in the expectorate and 5 mL water rinse. Subtraction of the total amount of zinc recovered in toothbrush wash, expectorate and 5 mL mouthrinsing from the known zinc dose gives a figure for oral zinc retention of 619 μg, equivalent to 38% of the dose of zinc in the 1 g of toothpaste used.

Elevated zinc levels were also detected in plaque and saliva samples taken after toothbrushing (Table 2). Plaque was sampled from the lingual aspect of the molars and zinc analyses are expressed per unit wet weight. It is therefore unknown whether the zinc so recovered was present intra- or extra-cellularly. Salivary zinc levels were raised throughout the 2 h sampling period. Results shown are the mean values for 10 subjects.

Table 1. The fate of zinc after toothbrushing with a 0·5% ZCT dentifrice. Results expressed in μg as mean ± s.e., n = 10.

Dose of zinc	1612·3 ± 36·4 μg
Zinc remaining on brush	154·3 ± 36·7 μg
Zinc expectorated and rinsed	839·1 ± 98·8 μg
Zinc retained	618·9 ± 82·1 μg
Zinc retained (as % of dose)	38·4%

Table 2. Zinc levels in plaque and saliva after use of a 0·5% ZCT dentifrice. Results expressed as mean μg zinc g^{-1} whole, unstimulated saliva or mean μg zinc mg^{-1} wet weight of plaque ± s.e., n = 10.

	Zinc level	
Plaque (5 min post brushing)	0·82 $\mu g\ mg^{-1}$	±0·37
Saliva: 5 min	7·6 $\mu g\ g^{-1}$	±1·2
Saliva: 30 min	3·1 μg	±0·4
Saliva: 60 min	2·2 $\mu g\ g^{-1}$	±0·3
Saliva: 120 min	1·1 $\mu g\ g^{-1}$	±0·2
Saliva (background)	< 0·2 $\mu\gamma\ g^{-1}$	
Plaque (background)	< 0·04 $\mu g\ mg^{-1}$	

Table 3. Zinc uptake by hydroxyapatite in-vitro.

Treatment	Zinc content of treated hydroxyapatite ($\mu g\ g^{-1}$)
0·132% zinc acetate	13 760
0·033% zinc acetate	13 620
0·013% zinc acetate	7380
Water	20
Extract of 0·5% ZCT toothpaste (10 g + 30 mL water)	7725
Paste extract diluted × 4	4575
Paste extract diluted × 10	3000

Table 4. Uptake of zinc to the pellicle-covered tooth surface from a dentifrice containing 0·5% zinc citrate.

Time of exposure (min)	Zinc uptake ($\mu g\ cm^{-2} \pm$ s.e.)
0·25	3·34 ± 0·10
0·50	4·15 ± 0·07
1·00	5·86 ± 0·20
5·00	7·45 ± 0·14
15·00	7·79 ± 0·18

Table 5. Desorption of zinc from the tooth surface.

Time (min)	Mean amount of zinc desorbed ($\mu g\ cm^{-2} \pm$ s.e. n = 6) Unlabelled zinc in saliva ($\mu g\ g)^{-1}$				
	0	1	2	5	10
5	4·51 ± 0·46	4·04 ± 0·17	2·55 ± 0·09	2·97 ± 0·12	2·04 ± 0·12
15	4·69 ± 0·32	4·09 ± 0·12	3·36 ± 0·20	3·26 ± 0·19	2·03 ± 0·16
30	4·54 ± 0·15	3·91 ± 0·09	2·81 ± 0·14	3·05 ± 0·13	2·24 ± 0·13

The levels of zinc taken up by hydroxyapatite in-vitro are shown in Table 3. These data demonstrate that zinc is available from an aqueous dentifrice extract to bind to hydroxyapatite in-vitro. A level many times in excess of background levels was acquired even from a 10-fold dilution of paste extract.

The results of the measurements of uptake of zinc from the 0·5% ZCT toothpaste to the pellicle-coated tooth surface are shown in Table 4. Uptake increased over the first 5 min of exposure, but little further uptake occurred over the remaining 10 min of the experiment, possibly indicating that the binding process was approaching saturation.

The desorption experiments were designed to test whether pellicle-bound radiolabelled zinc could subsequently desorb into saliva. Table 5 shows that desorption was greatest in the absence of added unlabelled zinc, and that as the concentration of unlabelled zinc in the saliva increased, the extent of ^{65}Zn desorption was reduced. The levels of unlabelled zinc in saliva were chosen to be similar to those measured in saliva in the period following toothbrushing with the 0·5% ZCT dentifrice.

Discussion

These data demonstrate the delivery of zinc to the mouth during toothbrushing with the toothpaste containing 0·5% ZCT. The oral retention of 38·4% of the dose of zinc is calculated taking no account of possible losses that may have occurred due to inadvertent swallowing of toothpaste. This has previously been estimated in model systems to be ca 5% of the dose (Birkeland & Lökken 1972).

Oral retention has also been demonstrated for other metal ion antiplaque agents. Bonesvoll & Rölla (1978) showed 37% of the tin in a 22·2 mmol L^{-1} stannous fluoride mouthwash to be retained in the mouth immediately after use. Similarly, 31% retention of copper was reported from a 100 mmol L^{-1} copper sulphate mouthwash (Afseth et al 1983a).

Zinc is taken up by plaque in-vivo. The levels reported in this study are similar to those reported elsewhere using a zinc acetate mouthwash (Afseth et al 1983b) and a little higher than those reported by Saxton et al (1986). It is likely that the presence of zinc in plaque, and bound to the dental pellicle, is partly responsible for the anticalculus and antiplaque effects observed with zinc citrate (Saxton et al 1986; Stephen et al 1987). The background level of zinc in plaque, determined before the study, was less than 0·04 μg Zn mg^{-1} wet weight, probably representing adventitious zinc from diet etc. This value is consistent with the finding of 0·103 μg Zn mg^{-1} dry plaque (Schamschula et al 1977) which would correspond to ~0·021 μg Zn mg^{-1} wet plaque.

Agents showing the ability to inhibit dental calculus formation adsorb upon hydroxyapatite crystallites and restrict their subsequent crystal growth (Francis 1969). The results in Table 3 show that zinc is acquired by hydroxyapatite from zinc acetate solution and from dilutions of a zinc citrate-containing toothpaste. In their procedure in which seeded crystal growth of hydroxyapatite in supersaturated calcium phosphate solution was monitored, Ingram & Carter (1987) found that pretreatment of hydroxyapatite with 0·035 and 0·0035% zinc acetate substantially inhibited subsequent crystal growth. The solution level of 0·013% zinc acetate described in Table 3 lies between these concentrations and gave a zinc uptake of over 7000 $\mu g\ g^{-1}$.

Dry plaque contains approximately 8 μg (Ca) mg^{-1} (Jenkins 1978). This corresponds to 20 μg of hydroxyapatite mg^{-1}. The level of zinc in wet plaque was 0·82 $\mu g\ mg^{-1}$, corresponding to approx 4 μg Zn mg^{-1} dry wt. On the basis of the amount of calcium phosphate present this would give an uptake of 200 mg Zn g^{-1} hydroxyapatite. Thus, although zinc may also be adsorbed elsewhere in the plaque, this figure of 200 mg Zn g^{-1} represents a 10–30 fold excess of zinc over those levels shown to be acquired by hydroxyapatite and to inhibit its crystal growth.

The tooth uptake studies show that zinc binds to the pellicle-coated tooth surface. Although these studies were carried out in-vitro they indicate that binding sites are unlikely to be saturated with zinc during a 1 min brushing period. The desorption experiments illustrate a potential mechanism by which zinc levels may remain elevated at the putative sites of anticalculus and antiplaque efficacy, i.e. the pellicle-coated tooth surface and plaque. The presence of elevated zinc levels in saliva reduced losses from the tooth surface compared with desorption into saliva with no added zinc. If the zinc is desorbed from teeth and bound to glycoproteins in the saliva, saturation or partial saturation of the glycoproteins by added zinc will reduce the 'sink' effect of the saliva.

The elevated zinc levels found in saliva after use of the dentifrice are probably a reflection of the desorption of zinc from intra-oral binding sites. Zinc binds to oral soft tissues (Gilbert & Baehni 1986) and it is likely that salivary clearance is responsible for its eventual removal from the mouth. However, this salivary zinc will, as the in-vitro studies indicate, reduce loss of zinc at the tooth surface and in the plaque.

In conclusion, these data demonstrate that zinc is retained in the mouth after toothbrushing with a 0·5% ZCT dentifrice. Uptake of zinc can occur at sites in plaque and on the pellicle-covered tooth surface. This mechanism may account for the reported anticalculus efficacy of the 0·5% zinc citrate dentifrice (Stephen et al 1987).

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