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The oral clearance of zinc and triclosan after delivery from a dentifrice

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Oral delivery and retention of zinc and triclosan were measured in man after use of an anti-plaque dentifrice containing these antibacterial agents. Triclosan (25% of the dose) and zinc (24%) were retained in the mouth immediately after toothbrushing and a single mouthrinse. Salivary concentrations fell from 7·5 μg g^{-1} (triclosan) and 7·0 μg g^{-1} (zinc) after 5 min to 0·5 μg g^{-1} (triclosan) and 1·2 μg g^{-1} (zinc), 2 h after brushing.

Recently, toothpastes have been developed using the suggestion of Hull (1980) that chemical control of plaque may be attempted. Their action is likely to rely on delivery of the anti-plaque chemical from the toothpaste to the oral cavity. In this way the chemical may increase the effect of brushing alone.

The anti-plaque efficacy of a dentifrice containing triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) and zinc citrate has recently been reported (Saxton 1986; Saxton et al 1987). The oral retention of these antibacterial agents from this dentifrice is now reported.

Oral retention has been reported for a number of anti-plaque agents. For example, Bonesvoll & Rölla (1978) showed that 37% of the tin in a 22·2 mmol L^{-1} stannous fluoride mouthrinse was retained in the mouth immediately after use. Values have also been reported for other metal ions: Afseth et al (1983) found 31% retention for copper and 15% for zinc after use of a mouthrinse containing 0·1 mol L^{-1} copper sulphate and a 2·5 mol L^{-1} zinc acetate mouthrinse. In a comparable study, Harrap et al (1984) demonstrated that 12% of the zinc from a 30 mmol L^{-1} zinc phenolsulphonate mouthrinse was retained in the mouth after expectoration of the rinse.

Studies of other potential anti-plaque agents have shown higher levels of retention with organic antibacterial compounds. These include 32% retention of chlorhexidine digluconate, 65% retention of cetylpyridinium chloride and 70% retention of hexadecyltrimethylammonium bromide (Bonesvoll & Gjermo 1978). The first of these compounds, chlorhexidine, has been widely studied and found to be 26–36% retained in normal subjects (Bonesvoll et al 1974; Gjermo et al 1974). In edentulous subjects, 19% of the dose was retained (Bonesvoll & Olsen 1974), indicating that there may be binding of the antibacterial to the teeth.

The aim of the current study was to measure the fate and retention of these antibacterial agents after delivery to the mouth during toothbrushing with a dentifrice containing 0·5% (w/w) zinc citrate and 0·2% w/w triclosan. Zinc citrate has been used as an antibacterial agent in toothpastes, and triclosan is a non-ionic antibacterial compound with a wide spectrum of activity (Vischer & Regos 1974) which has been used in skin preparations. Its formulation with zinc in a toothpaste has led to an anti-plaque effect which is thought to be due to delivery of these active agents to the oral cavity during toothbrushing (Gilbert & Williams 1987).

Materials and methods

Toothpaste. The toothpaste contained two agents present for their antibacterial activity. These were triclosan (Ciba Geigy, Manchester, UK) (0·2%, w/w), and zinc citrate trihydrate (0·5%, w/w) (Sturge, Birmingham, UK). The anticaries agent, sodium monofluorophosphate (0·85%, w/w), alumina abrasive (50·00%, w/w), detergent (2·50%, w/w), water (16·17%, w/w) and other excipients (humectant, flavour, colour) comprised the remainder of the toothpaste formulation.

Experimental protocol. Ten volunteers aged 20–40 participated in the study, and comprised seven females and three males. They were selected to exclude prospective volunteers who were pregnant, on antimicrobial therapy or who had overt dental disease. Each volunteer used the toothpaste twice, both for measurement of oral retention, once additionally followed by measurement of saliva clearance (procedure 1, below) and once by subsequent recovery of antibacterial agents (procedure 2, below). In both procedures, participants were asked not to brush their teeth during the 24 h preceding the test.

Sampling procedures

Procedure 1. Using a wet toothbrush, volunteers brushed their teeth for 1 min with 1 g of the toothpaste, accurately weighed. A new toothbrush was used on each occasion. The brush and expectorate were collected and a 30 s 5 mL water rinse then carried out. Finally, unstimulated saliva samples were taken by expectoration 5 min, 30 min, 1 and 2 h after brushing.

Procedure 2. The brushing regime was exactly as above. After the water rinse, however, volunteers rinsed with 3 successive 10 mL volumes of an oral mouthrinse formulated to recover triclosan and zinc retained on the oral surfaces during toothbrushing. This rinse comprised an aqueous solution containing 15% (v/v) ethanol, 0·2 mol L^{-1} glycine and 0·001 mol L^{-1} sodium hydroxide (pH = 9·0).

Sample analyses

Triclosan. Samples were collected into pre-weighed 30 mL screw top glass vials and the weight of sample recorded. A weighed portion of the sample (ca 2·0 g) was mixed in a 15 mL centrifuge tube with 2·0 mL 96% ethanol and 0·2 mL perchloric acid (20% v/v), to precipitate salivary proteins. After mixing, the sample was stood on ice for 15 min, and was then further mixed and centrifuged at 3500 *g* for 15–20 min to concentrate the precipitated protein to a pellet. A portion of the supernate was collected into a glass vial and analysed as described below. The toothbrush was washed in ca 25 g of ethanol, which was weighed and a portion analysed as described below. Triclosan was measured by HPLC. A series 5000 liquid chromatograph (Varian Ass. Palo Alto, California, USA) delviered eluant at a constant 2 mL min^{-1} to a 15 cm × 46 mm reversed phase silica column (Hypersil 5 μm ODS, HPLC Technology Ltd, Macclesfield, UK). The eluant was an aqueous phosphate-buffered acetonitrile mixture of pH 3·0. This comprised 0·1 mol L^{-1} phosphoric acid–0·1 M disodium hydrogen phosphate–acetonitrile–water (4:1:10:10, v/v). Peak areas were analysed using a Varian 4270 integrator. Triclosan recovery was greater than 95% in samples containing greater than 10 μg triclosan g^{-1} saliva, and greater than 85% in samples containing 0·5–10 μg triclosan g^{-1} saliva.

Zinc. The principle (Delves 1981) of acid extraction of zinc from samples containing protein was used. Portions (2·0 g) of each sample 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.

Analysis 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 μg 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%.

Calculation of kinetic parameters

For each volunteer, data for levels of triclosan and zinc in saliva at various time points was fitted to the following model

$$C_s = C_o\, e^{K_{el}t}$$

where C_s = concentration of antibacterial agent in volunteer's saliva at time t, C_o = theoretical zero time concentration of antibacterial agent in volunteer's saliva, K_{el} = elimination constant for antibacterial agent.

The elimination constant K_{el} and theoretical zero time saliva concentrations for individual volunteers were estimated via linear regression (Armitage 1971) after applying natural logarithm transformation to both sides of the model's equation i.e.

$$\text{Ln } C_s = \ln C_o - K_{el}t$$

The biological-half life ($t_{\frac{1}{2}}$) of the antibacterial agents in saliva was calculated using the equation $t_{\frac{1}{2}} = 0{\cdot}693/K_{el}$.

Results

Oral retention of triclosan and zinc. Analyses of the material on the toothbrush, in expectorate and in mouthrinsings show that 22% of the original triclosan dose was present in the toothpaste remaining on the toothbrush after use (Table 1). The equivalent figure for zinc was 9% of the dose.

Table 1. Oral retention of triclosan and zinc after 1 min toothbrushing and a 30 s 5 mL water rinse (mean and s.e. n = 20). Figures in parentheses represent these quantities expressed as a percentage of the dose.

	Triclosan		Zinc	
	Mean	s.e.	Mean	s.e.
Dose of antibacterial (μg)*	2134	± 71	1665	± 52
Quantity recovered on brush (μg)	472(22%)	± 44	149(9%)	± 58
Quantity expectorated (μg)	1021(48%)	± 71	976(59%)	± 56
Quantity rinsed out (μg)	103(5%)	± 06	138(8%)	± 13
Oral retention (μg)	538(25%)	± 83	402(24%)	± 65

* The mean weight of paste used was 1·067 g.

The expectorate contained 48% of the triclosan and 59% of the zinc dose. After water rinsing, about one quarter of the original dose of triclosan and zinc was retained in the mouth. These figures are derived by subtraction and therefore take no account of the loss that may have occurred due to inadvertent swallowing. This loss has previously been estimated by Birkeland & Lökken (1972), using model systems, to be ca 5% of the original dose.

Triclosan and zinc levels in saliva. Salivary levels of triclosan and zinc found to be present from 5 min to 2 h after brushing are shown in Table 2. Triclosan was present in saliva for at least this 2 h period; zinc levels were also raised significantly above background during this time. Table 2 also shows pharmacokinetic parameters calculated from these data.

Triclosan and zinc recovered by glycine ethanol mouthrinse. The purpose of using this mouthrinse was to recover triclosan and zinc which had been deposited on the oral mucosa during toothbrushing. The quantities of

Table 2. Pharmacokinetic analysis of triclosan and zinc levels in saliva (n = 10).

Time (min)	Mean saliva level ($\mu g\ g^{-1}$, range)	Elimination constant ($K_{el} \pm$ s.e., min^{-1})	Biological half-life ($t_{\frac{1}{2}} \pm$ s.e., min^{-1})	Mean theoretical zero time saliva concn ($\mu g\ g^{-1}$, range)
		Triclosan		
Background	0			
5	7·5 (3·7–15·0)			
30	3·4 (1·9–6·2)	0·035 ± 0·003	20·0 ± 2·0	8·5 (3·6–22·4)
60	1·3 (0·1–4·5)			
120	0·5 (0·05–1·6)			
		Zinc		
Background	<0·2			
5	7·0 (3·0–15·8)			
30	4·6 (1·8–8·2)	0·0147 ± 0·001	47·0 ± 5·0	6·0 (3·0–13·0)
60	2·5 (0·8–3·4)			
120	1·2 (0·5–2·0)			

Table 3. Triclosan and zinc recovered by glycine/ethanol mouthrinse after toothbrushing. Results expressed are the mean amount of antibacterial agent recovered in each of three successive 1 min oral mouthrinses ($\mu g \pm$ s.e., n = 10).

	Triclosan	Zinc
First rinse	35·1 ± 3·4 μg (1·6%)*	65·1 ± 3·4 μg (3·9%)
Second rinse	23·6 ± 5·4 μg (1·1%)	15·2 ± 2·2 μg (0·9%)
Third rinse	17·5 ± 2·5 μg (0·8%)	14·6 ± 3·9 μg (0·9%)
Total recovered	3.5%	5·7%

* Figures in parentheses are the percentage of the initial antibacterial dose.

the antibacterial agents recovered are shown in Table 3. The total recoveries, expressed as a percentage of the dose of the antibacterial concerned, are 5·7% for zinc and 3·5% for triclosan. These figures represent 24% and 14%, respectively, of the overall levels of antibacterial calculated to have been retained in the mouth on the basis of the mass balance calculations.

Discussion

These results show that about one quarter of the dose of triclosan and zinc are retained in the mouth after toothbrushing with the test dentifrice. This level of retention is similar to published figures for chlorhexidine (Bonesvoll et al 1974; Gjermo et al 1974; Bonesvoll & Gjermo 1978). While there have been no previous reports of the mass balance of overall oral zinc retention following toothpaste use, the observed value of 24% retention in this study is greater than the values of 15 and 12% reported following use of zinc-containing mouthwashes (Afseth et al 1983; Harrap et al 1984).

In the current study, new toothbrushes were used on each occasion of brushing. Two factors probably contribute to the recovery of antibacterial agents on toothbrush, one relating to the amount resident in the toothpaste left on the brush and the other possibility that the agents actually bind to the toothbrush material. The recovery of different levels of triclosan and zinc from the toothbrush may be due to extra binding of triclosan to the brush material with similar recovery of both agents from the toothpaste remaining on the brush.

Levels of zinc found in saliva (Table 2) are similar to those reported elsewhere following use of a toothpaste containing 0·5% (w/w) zinc citrate (Saxton et al 1986), and following use of a mouthrinse containing zinc (7·5 mg) and hexetidine (Saxer & Mühlemann 1983). These studies did not measure the extent of overall oral retention of zinc.

The measured levels of zinc and triclosan in saliva follow first order kinetics over the 2 h after toothbrushing. The median theoretical zero time concentrations calculated from these data are 8·5 μg triclosan g^{-1} saliva and 6·0 μg zinc g^{-1} saliva. The ratio of 8·5 : 6·0 $\mu g\ g^{-1}$ is 1 : 0·71 which is similar to the ratio of the amounts of these antibacterial agents calculated to be retained in the mouth (538 μg : 402 μg, i.e. 1 : 0·75, Table 1). This similarity may be a reflection of similar retention mechanisms for these agents in the mouth.

The calculated values for the biological half-life of triclosan (20·0 min) and zinc (47·0 min) in saliva suggest that triclosan is cleared more quickly from the mouth than zinc. The determination of efficacy of these antibacterial agents is likely to rely on the relationship between concentration of antibacterial agent and time of exposure required to reduce growth of oral bacteria.

Triclosan is very soluble in ethanolic solution and ethanol was included in the mouth rinse to recover as much triclosan and zinc from the mouth after toothbrushing as possible. That the quantity of antibacterial agents actually recovered (Table 3) is much lower than the amount calculated to be retained (Table 1) may be due to a number of reasons. The most likely cause is that triclosan binds to protein and additionally is lipophilic. It would be expected to be taken up by the oral mucosa; indeed this reservoir is probably the main source of salivary triclosan during the post-brushing period.

It is likely that the clinical anti-plaque activity reported for this toothpaste (Saxton 1986; Saxton et al 1987) is due to the retention of triclosan and zinc at specific sites of activity in the mouth.

In conclusion, about one quarter of the triclosan and zinc present in an anti-plaque toothpaste is retained in the mouth. Both these antibacterials are present in saliva for at least 2 h after brushing.

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Antiarrhythmic effect of desethylamiodarone in conscious rats

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The effect of desethylamiodarone, a metabolite of amiodarone, was studied in the early phase of arrhythmias induced by coronary artery ligation in conscious rats. Desethylamiodarone pretreatment improved survival without altering the occurrence of different types of arrhythmias during the first 20 min after coronary ligation. It was concluded that desethylamiodarone may contribute to the antiarrhythmic effect seen after chronic amiodarone treatment.

Amiodarone is effective in the treatment of cardiac arrhythmias especially after chronic administration. It is also more effective against experimental arrhythmias in dog (Patterson et al 1983) and rat (Varró & Rabloczky 1986) after chronic, compared with acute treatments. During chronic treatment, the metabolite, desethylamiodarone, appears in plasma and accumulates in cardiac muscle (Plomp et al 1985). Electrophysiological studies indicate that desethylamiodarone delayed repolarization of cardiac muscle both in-situ (Nattel 1986) and in-vitro (Yabek et al 1986). However, no data are available so far about the antiarrhythmic effect of desethylamiodarone even though it is likely that the effects of desethylamiodarone contribute to the strong antiarrhythmic efficacy of the chronic amiodarone administration. We describe here the antiarrhythmic action of desethylamiodarone in conscious rats.

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Methods

The method of Leprán et al (1983) was used. Young male rats, 200–250 g, were anaesthetized by ether inhalation.

After thoracotomy, a loose loop of thread was placed around the descending branch of the left coronary artery. The end of the loop was exteriorized through a polyethylene tube. Thereafter, the chest was closed. Artificial ventilation was not needed during the entire surgical manoeuvre. The ends of the thread were hidden subcutaneously. Seven to ten days after that operation, the thread was exteriorized under light ether anaesthesia and the loop tightened, thereby producing acute myocardial ischaemia. ECG recordings were taken by a bipolar thoracic lead both before and continuously for 20 min after ligation (Medicor ER 362). The occurrence of several types of arrhythmias and the survival ratio were determined.

The post-ligation arrhythmias were categorized as ventricular extrasystole (single, double or triple premature beats), bradycardia (heart rate lower than 200 beats min^{-1}, typical signs of A–V block), ventricular fibrillation (very high frequency potentials with irregular amplitudes) as was suggested by Leprán et al (1983).