The oral retention and antiplaque efficacy of triclosan in human volunteers

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- 1 An antiplaque toothpaste containing 0.2% (w/w) triclosan significantly inhibited overnight plaque growth in 12 human volunteers.
- 2 After use of a 1 g quantity of the toothpaste containing 0.2% (w/w) [^3H]-triclosan, $36.3 \pm 1.4\%$ of the triclosan was retained.
- 3 The mean concentration ($\pm$ s.d.) achieved in saliva 5 min after toothpaste use was $8.16 \pm 2.50 \mu\text{g g}^{-1}$.
- 4 The saliva decay curve for triclosan was consistent with a two phase model with $t_{1/2}$ values of 0.45 h and 2.42 h, respectively.
- 5 Triclosan was present in bacterial plaque for at least 8 h after dosage and in the oral mucosa for at least 3 h.

Keywords triclosan dentifrice antiplaque agent

Introduction

There have been a number of recent reports of the antiplaque efficacy of a toothpaste containing 0.2% (w/w) triclosan (Saxton, 1986; Saxton *et al.*, 1986). This compound (2-4-4'-trichloro-2'-hydroxy diphenyl ether), is an antibacterial agent with a wide spectrum of activity (Vischer & Regos, 1974) and has been widely used in various personal products. This paper describes measurements of the oral retention and pharmacokinetics of [^3H]-triclosan delivered from a toothpaste in healthy human volunteers.

Chemical control of dental plaque (in addition to mechanical plaque removal by brushing) was suggested by Hull (1980). There are several examples of studies in humans of oral retention of substances intended to have an antiplaque effect. Most of these studies have been carried out using a mouthrinse as the delivery vehicle. Bonesvoll & Rølla (1978) showed 37% of the tin in a 10 ml 22.2 mM SnF_2 mouthrinse to be retained. Similar values have been reported for

other metal ions. Thus, Afseth *et al.* (1983) found 31% retention of copper and 15% of zinc after use of a mouthrinse containing 0.1 mol l^{-1} copper sulphate and 2.5 mol l^{-1} zinc acetate. In a comparable study, Harrap *et al.* (1984) demonstrated 12% retention from a 30 mM zinc phenol-sulphonate mouthwash.

Studies of other potential antiplaque 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 has been widely studied and found to be 26%–36% retained in normal subjects (Bonesvoll *et al.*, 1974; Gjermo *et al.*, 1974).

A previous investigation (Gilbert *et al.*, 1987) of the retention and oral pharmacokinetics of triclosan showed 37% of the dose to be retained. A higher dosage level (0.5%, w/w) of triclosan

was used than in the current study and triclosan concentrations in saliva and plaque were detectable by h.p.l.c. up to 2 h after dosage.

The current work was carried out to allow measurements to be made for up to 8 h and to permit repeated plaque and buccal mucosa sampling to be made.

Methods

Study approval

The work was carried out in accordance with the provisions of the Declaration of Helsinki and Tokyo Revisions (1975). Approval was received from the Simbec Independent Ethics Committee and was subject to periodic review by that committee. Permission to undertake the work was received from the Administration of Radioactive Substances Advisory Committee of the D.H.S.S in the form of certificate No. RPC 331-2(4).

Volunteers

Twelve healthy male subjects aged 19–37 years were selected. Each had a least 16 natural teeth, no overt caries and no large restorations in the region of the gingival margin. All volunteers were subjected to a full clinical examination with sample analysis for haematology, clinical chemistry and urinalysis. All gave written informed consent. Volunteers did not brush their teeth for 48 h before each part of the test.

Clinical plaque growth measurement

The inhibition of plaque growth due to brushing with the triclosan and zinc citrate containing dentifrice was measured relative to a placebo by the Gingival Margin Plaque Index method of Harrap (1974). In brief, participants first brushed their teeth thoroughly with a simple abrasive paste to remove as much plaque as possible. The purpose of this step was to ensure that both parts of the plaque growth test were carried out from a low baseline plaque score for each individual. The teeth were then brushed with about 1.0 g of test or placebo dentifrice for 1 min. The disclosing dye erythrosin (0.5%) was applied to the teeth to assess any remaining plaque using the gingival margin plaque index (Harrap, 1974).

Any form of oral hygiene was prohibited until after the plaque was reassessed approximately 16 h later. Plaque regrowth was defined as the difference between the two plaque assessments. The participants used both the test and placebo

dentifrices 3 days apart and in random order. No carryover of triclosan or zinc could be detected between applications of the test dentifrice 3 days apart (C. A. Saxton, personal communication).

Toothpaste formulation and dosage

Two test toothpastes were used. These contained 0.2% (w/w) triclosan and 0.5% (w/w) zinc citrate trihydrate together with sodium monofluorophosphate, anionic surfactant, humectant, binder, water, minor excipients and aluminium trihydrate abrasive. The test paste for the plaque growth brushing test contained unlabelled triclosan while that used in the pharmacokinetic study contained tritiated triclosan of specific activity $38.6 \mu\text{Ci mg}^{-1}$ (6,6-[^3H]-2,4,4'-trichloro-2'-hydroxy-diphenyl ether). This was obtained from CIBA-Geigy (Manchester, UK), tritiated at Amersham International (Cardiff, UK) and purified using ion-exchange chromatography. The position of tritiation was confirmed by tritium NMR, radio t.l.c. and h.p.l.c. and the material was found to be greater than 98% pure (T.C. Pestell, personal communication).

For the pharmacokinetic work, the toothpaste was dosed as a thoroughly mixed slurry of 1 g paste in 4 ml water. This was rinsed around the mouth for 1 min and expectorated.

Sample collection and analysis

The dosage expectorate, a further 30 s water mouthrinse (10 ml) and toothpaste slurry remains from the dosage vessel were analysed for [^3H]-triclosan. Unstimulated saliva samples were collected by expectoration at 0.05, 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0 and 8.0 h after dosage. Plaque samples were collected into preweighed vials by use of a dental scraper 0.25, 1.0, 3.0 and 8.0 h after dosage and scrapings of buccal mucosa taken after 0.25, 1.0 and 3.0 h.

All samples were analysed in duplicate as follows. Samples containing toothpaste, (the paste itself, expectorate and rinsings) were extracted with 2.0 ml methanol and 0.2 ml portions of the extract were dried in combustion cones prior to oxidation in a Packard B-306 Oxidiser. Saliva was applied directly to combustion cones and dried for scintillation spectroscopy as above. Plaque and buccal mucosa samples were homogenised in phosphate buffer (pH 7.0) by ultrasonication. This sonicate was divided into two weighed portions: one was assayed for protein content according to the Biorad method (Bradford, 1976) and the other dried and oxidised. Measurements of radioactivity by scintillation

spectroscopy were calibrated using a methanolic solution of [^3H]-triclosan ($38.6 \mu\text{Ci mg}^{-1}$) and quench correction was by the external standard channels ratio method. Addition of known amounts of triclosan indicated that triclosan recovery was greater than 95% for samples of toothpaste slurry, expectorate and mouthrinse, and greater than 90% for all other samples. The minimum amount of triclosan detectable using this radiochemical procedure was 10 ng.

In subsequent calculations it was assumed that all measured tritium was present as [^3H]-triclosan.

Results

The antiplaque activity of the toothpaste formulation was measured using the non-radiolabelled dentifrice. The mean plaque growth ($\pm$ s.d.) with the test paste was 11.3 ± 6.2 units compared with growth using the placebo paste of 23.1 ± 7.9 units. This difference was significant at the 1% error probability level, using the Student's paired *t*-test (Armitage, 1971).

After measurement of ^3H -levels remaining in the dosage vessels and in the expectorate and rinse, the oral retention of triclosan was calculated by difference (Table 1). The mean oral retention of $873.3 \mu\text{g}$ triclosan was equivalent to 36% of the 1 g toothpaste dose. The 10 ml mouthrinse removed $4.1 \pm 0.9 \mu\text{Ci}$ of tritium (equivalent to 4.4% of the triclosan dose).

The levels of triclosan measured in saliva after toothpaste use are shown in Figure 1. At least 1 g of saliva was collected at each sampling. The time needed for collection of a single saliva sample was usually about 2 min.

Triclosan was present for at least 8 h after dosage. The level of radioactivity detected after 8 h was equivalent to $0.08 \mu\text{g}$ triclosan g^{-1} saliva.

Figure 1 Levels of radioactivity in saliva after mouth-rinsing for 1 min with 1 g of toothpaste in 4 ml water. The toothpaste contained 2 mg g^{-1} [^3H]-triclosan (specific activity $38.6 \mu\text{Ci mg}^{-1}$) and dosing was followed by expectoration and a 10 ml, 30 s mouthrinse. Saliva samples were combusted and tritium measured by scintillation spectroscopy. All radioactivity is assumed to be in the form of [^3H]-triclosan, each point represents the mean of 12 determinations, except those of 0.28 and 1.00 h ($n = 11$). Standard deviations are shown in Table 3.

Measurement of radioactivity in buccal mucosa and plaque showed activity to be present throughout the measurement periods of 3 h and 8 h, respectively. The triclosan levels in these samples are shown in Table 2.

Discussion

The antiplaque effect demonstrated by the significant difference in plaque growth at the gingival

Table 1 Disposition of [^3H]-triclosan after 1 min mouthrinsing with 4 ml toothpaste slurry containing 1 g of toothpaste. Results expressed as mean $\pm$ s.d. ($n = 12$). The mean weight of paste used was 1.034 ± 0.027 g, equivalent to $2404.9 \pm 62.7 \mu\text{g}$ triclosan

	^3H (μCi)	Triclosan (μg)
Dose	92.8 ± 2.42	2404.9 ± 62.7
Remaining in dosing vessel	21.8 ± 9.3	564.5 ± 240.4
Expectorated	30.7 ± 7.3	795.8 ± 187.8
Rinsed out	4.1 ± 0.9	105.2 ± 22.7
Minor losses*	2.5 ± 0.7	66.1 ± 16.3
Retained dose	33.7 ± 3.5	873.3 ± 90.4

*Minor loss comprises spillage during dosage, activity recovered from foil caps of dosage vessel, etc.

Table 2 Levels of [^3H]-triclosan found in plaque and oral mucosa at various times after dosage with 4 ml toothpaste slurry containing 1 g of toothpaste. Results are expressed as mean $\pm$ s.d. ($n = 12$)

Time after dosage (h)	Plaque triclosan level ($\mu\text{g mg}^{-1}$ protein)	Buccal mucosa triclosan level ($\mu\text{g mg}^{-1}$ protein)
0.25	1.20 $\pm$ 0.38	4.12 $\pm$ 2.24
1	0.50 $\pm$ 0.30	0.89 $\pm$ 0.80
3	0.21 $\pm$ 0.14	0.21 $\pm$ 0.14
8	0.10 $\pm$ 0.05	n.d.

n.d. = not determined.

margin relative to a placebo toothpaste is consistent with the antiplaque efficacy of this dentifrice formulation reported elsewhere (Saxton, 1986; Saxton *et al.*, 1987). The purpose of the work described in this paper was to demonstrate delivery of triclosan to the mouth during toothpaste use. The levels of this antimicrobial agent found in the mouth are likely to be a major factor in determining the observed antiplaque efficacy.

Oral retention of 36% of the triclosan dose is similar to the value of 37% previously reported for triclosan retention from a 0.5% w/w triclosan toothpaste slurry (Gilbert *et al.*, 1987). The levels of triclosan retention, calculated by difference, are similar to values reported for the widely used oral antibacterial agent chlorhexidine. This compound has been found to be 26%–36% retained in normal human subjects (Bonesvoll *et al.*, 1974; Gjermo *et al.*, 1974).

No attempt has been made in the current work to investigate the possibility of salivary metabolism of the triclosan molecule. All radio-

activity recovered has been assumed to be in the form of unaltered triclosan.

The levels of triclosan found in saliva immediately after toothpaste use were similar to those reported elsewhere after measurement by h.p.l.c. (Gilbert, 1987). It is likely that these concentrations are due to desorption of triclosan from the oral soft tissues. The longer time of detection in the current study allows pharmacokinetic analysis of the triclosan decay profile. The saliva decay plot (Figure 1) is consistent with biphasic behaviour over the whole 8 h period. The two phases have $t_{1/2}$ values of 0.45 h and 2.42 h, respectively (see Table 3). Some evidence is available suggesting that different areas of oral epithelium and epidermis have different permeabilities to water and lipid soluble molecules (Moffatt, 1971; Squier & Hall, 1982). However, further work is necessary to establish whether such permeability differences can account for the two phases of saliva triclosan concentration observed in the current work.

Table 3 [^3H]-triclosan saliva levels, half life, rate constants and theoretical zero time saliva concentrations following use of toothpaste containing 0.2% (w/w) triclosan

Time after dosage (h)	Saliva triclosan concentration ($\mu\text{g g}^{-1}$)	Elimination constant (h^{-1})	Biological half-life (h)	Theoretical zero time saliva concentration ($\mu\text{g g}^{-1}$)
0.05	8.16 $\pm$ 2.50			
0.283	5.40 $\pm$ 1.91			
0.50	3.37 $\pm$ 1.68	1.54 $\pm$ 0.21	0.45	7.07
1.0	2.18 $\pm$ 1.25			
2.0	0.77 $\pm$ 0.52			
3.0	0.46 $\pm$ 0.24			
4.0	0.25 $\pm$ 0.19	0.28 $\pm$ 0.04	2.42	0.86
6.0	0.15 $\pm$ 0.13			
8.0	0.08 $\pm$ 0.05			

Saliva triclosan levels are the mean of 11 $\pm$ s.d. Kinetic parameters were calculated from these mean data. The data from one of the 12 volunteers have been excluded due to incomplete sampling.

The triclosan level measured in plaque 15 min after dosage is similar to that measured 5 min after rinsing with a triclosan dentifrice (Gilbert *et al.*, 1987). Although the variation in the plaque measurements precludes a meaningful calculation of the $t_{1/2}$ of triclosan in this oral microreservoir, a comparison with the saliva decay curve reveals a greater fall over 8 h from the 15 min triclosan level in saliva than was the case in plaque. This is a reflection of the reduced loss of triclosan from plaque compared with loss from buccal mucosa (Table 2). The loss from mucosa is likely to be the major factor determining saliva triclosan levels.

Our findings show that the antibacterial agent triclosan is retained in the mouth, particularly

in plaque, after dosing from a toothpaste and suggest that this oral deposition may be responsible for the observed antiplaque efficacy of this dentifrice.

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