Research paper

In vitro investigation of the liberation of fluoride ions from toothpaste compounds in a permeation model

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Abstract

The most important requirement for effective topical fluoride prophylaxis from toothpaste containing fluoride is that the active fluoride agent must be chemically free, and the rapid spread of the dissolved fluoride ions over the tooth surface. Abrasive compounds in the toothpastes and the brief residence time at the site of action, the oral cavity and tooth surface must not prevent the liberation. Using a two-chamber diffusion cell and an ion-selective fluoride electrode, the content of fluoride ions in five different fluoride-containing toothpastes was determined by direct potentiometry as a function of time and the different abrasive compounds employed. The investigation has demonstrated that a reduction of the release rate of fluoride ions by nearly 50% is seen when calcium carbonate and calcium hydrogen phosphate dihydrate are used as abrasive compounds and combined with sodium fluoride. © 1999 Elsevier Science B.V. All rights reserved.

1. Introduction

Few comparative liberation investigations of fluoride from toothpastes are to be found in the literature [1]. Forsman and Ericsson [1], Einwag [2] and Ellingsen and Ekstrand [3] in their studies show the absorption-inhibiting action of calcium carbonate and dicalcium phosphate dihydrate, which are used as abrasive compounds in toothpastes in the stomach after inadvertently swallowing toothpaste containing fluoride.

It is considered proven that fluoride ions from the surface of the tooth enamel, the hydroxylapatite, are absorbed with the formation of acid-resistant hydroxylfluorapatite [4].

However, a precondition for effective caries prophylaxis through regular local application of toothpaste containing fluoride is, that the fluoride ions must be present in sufficient concentration and freely available [5]. This is only possible if one takes account of the cleaning utensil used [6].

It can be expected, when using (1) calcium carbonate or (2) dicalcium phosphate as abrasive compounds in fluoride-containing toothpastes, that poorly soluble calcium fluoride will be formed.

The caries-protective action of the toothpaste would in this case be lost or drastically reduced. The use of a 20–60% abrasive compound content which is customary today could affect the liberation of fluorides and therefore the tooth surface fluoride uptake.

In addition, a maximum liberation toothpastes is required in order to provide the following effects: (1) aid posteruptive maturation of the enamel surface; (2) reduce enamel solubility and increase enamel resistance; (3) prevent demineralization; (4) enhance remineralization of incipient lesions; (5) alter the action of plaque bacteria [7,11].

The aim of the study was to test the liberation of fluoride ions from the different toothpaste compounds.

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It is important to note in this respect that only with an optimal combination of auxiliary substances with fluorides can a permanently effective galenic preparation effective for protection against caries be created.

For this purpose a special model system was developed. Attempts were made to create conditions approximating to in vivo conditions through the appropriate selection of temperature and time.

2. Materials and methods

2.1. Materials

The measuring electrode used was the fluoride-selective electrode type 152153000, from Mettler Toledo, Switzerland, with the reference electrode InLab® type 302 from Mettler (Toledo, Switzerland). A potentiometric pH meter CG 818-Digital from Schott type 213959 Schott (Hofheim, Germany) was employed. The dialysis tubing was Nadir® No. 69917020 from Roth (Karlsruhe, Germany).

The two-chamber diffusion cell according to Dibbern [15] was of in-house construction by the School of Pharmacy of the University of Halle, Wittenberg, Germany. TISAB IV® (Total Ionic Strength Adjustment Buffer) was batch 2E008A from Mettler Toledo (Steinbach, Germany). Sodium fluoride p.a. was from Merck, No. 1.59718 (Merck Darmstadt, Germany). Fluoride-containing formulations tested are shown in Table 1.

2.2. Methods

The determination of fluoride is carried out by direct potentiometry using an LaF_3 single-crystal ion-selective membrane electrode for the measuring range $<10^{-6}$ mol/l of fluoride up to fluoride saturation. A calibration curve is plotted by serial dilution of a concentrated stock solution of 1000 p.p.m. of fluoride from dried sodium fluoride to 100 p.p.m., 10 p.p.m., 1 p.p.m. and 0.1 p.p.m. (reference standard).

According to Hanocq et al. [9], the potentiometric method can be equated to the spectrophotometric method.

The in vitro liberation model used is a measuring cell modified according to the membrane model of Dibbern (Fig. 1). Using magnetic stirrers, provision is made for uni-
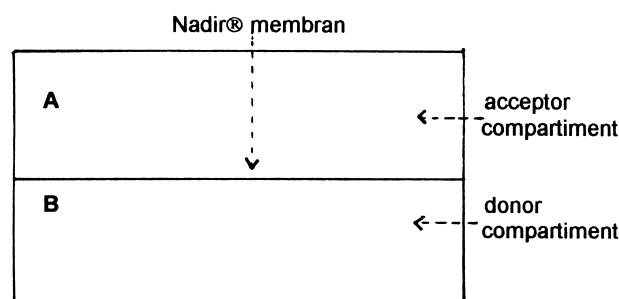

Fig. 1. Sketch of a membrane model of Dibbern (with Plexiglas 9×7 cm), including a removable membrane window for the insertion of a double membrane as a diffusion barrier.

form and adequate mixing of the solution, largely in order to prevent the build-up of a diffusion layer on the membrane. The constant temperature of 30°C and the same stirring conditions (i.e. identical stirring speed, identical stirring bars, identical distance between electrodes and stirring bars) is continuously monitored. The membrane used for the removable membrane window is cellulose hydrate tubing (Nadir® membrane), with the function of a diffusion barrier in the measuring cell.

In each case, 10.0 ml of analysis solution containing 500 mg toothpaste + 1.0 ml TISAB® (Total ionic strength adjustment buffer) and 10.0 ml distilled water are treated under the same conditions as used for plotting the calibration curve, and added to the donor compartment after mixing well at a constant stirring speed, measuring sequence and measuring time interval of 3 min for each measurement.

All experiments were carried out under the same conditions. After immersing the fluoride-selective electrode and the reference electrode, the constant final value was read off in millivolts, and using the calibration diagram the fluoride concentrations of the sample solutions were determined.

The TISAB® solution is a reagent consisting of an acetic acid/acetate buffer and cyclohexanediaminetetraacetic acid (CDTA) to release fluoride from hydrofluoric acid and for the release of the fluoride which complexes the aluminium, iron or other cations.

In order to ensure free determinations of total fluoride content, the pH value of both calibration and sample solutions must lie between 5 and 8, and the ionic strength of cell solutions concerned approximately equal.

The addition of TISAB® will adjust the pH value to

Table 1

Preparations tested with the active fluoride ingredient contained in each case and the accompanying cleaning compound as well as the manufacturer

Formulation	Batch	Fluoride active ingredient	Cleaning compound	Manufacturer	Country
A	597510	Olaflur/Hetaflur	Metaphosphate	Wybert	Germany
B	650606	Olaflur	Fine-grain poly-ethylene powder, silica	Wybert	Germany
C	6354R3	Sodium monofluoro-phosphate	Calcium carbonate	Procter and Gamble	UK
D	310297L	Sodium fluoride	Calcium hydrogen phosphate dihydrate	Blendax	Germany
E	223605	Sodium fluoride	Aluminium oxide dihydrate	Wybert	Germany
Gel	027607	Olaflur/Dectaflur		Wybert	Germany

between 5 and 8 and set the ionic strength to about 0.5 ml/l.

Interfering ions, as influenced by the composition of toothpaste, like high concentrations of lanthanum complexing anions (such as citrate, phosphate and bicarbonate), will impair the response characteristics.

The investigation of the content of fluoride ions in various toothcleaning preparations was carried out on the commercially obtained products A–E with different abrasive compounds comparing: concentration of fluoride ions without membrane (liberation test), concentration of fluoride ions using a membrane, the task of which should be seen as its simulation of the diffusion ratio in the mucous membrane of the mouth, and in order to demonstrate the extent to which fluoride ions can be resorbed within the usual teeth-cleaning period of approximately 3 min (systemic uptake) under otherwise identical conditions (temperature 30°C, TISAB 1.0 ml, 500 mg toothpaste and 10.0 ml distilled water, constant stirring speed, time interval of 3 min for measuring).

The content of fluoride ions determined by the potentiometric method thus gives information about the concentration of active fluoride ions measured without a membrane, or information regarding systemical uptake can be obtained by measuring with the Nadir® membrane in the case of an average tooth cleaning time of 3 min under constant conditions: temperature 30°C, 0.50 g toothpaste, 1.0 ml TISAB®, and 10.0 ml distilled water, $n = 3$, time interval 3 min, constant stirring speed, mean ± SD (2.9%) (Table 2).

The investigation takes no account of storage time losses and possible extreme temperature stresses. None of the preparations, however, were older than 10 months at most.

3. Results

Testing the various preparations based on aluminium oxide dihydrate (preparation E), as well as those based

Table 2

Fluoride content (%) in the various commercially available formulations A–E and gel

Formulation	Amount (g)	%F theoretical	% F measured (actual)	Total % F-
A	0.5	125	119 ± 4.1	95.2
B	0.5	140	118 ± 3.9	84.3
C	0.5	130	78 ± 1.8	74.0
D	0.5	120	62 ± 2.1	51.7
E	0.5	136	120 ± 0.8	88.2
Gel	0.3	1250	1119 ± 4.9	89.5

Content of 10.0 ml solution: quantity 0.5 g, 1.0 ml TISAB®; temperature 30°C, constant stirring speed, $n = 3$, time interval 3 min, ±2.9% SD, measured without membrane in the measuring cell (liberation test). Percentage F-theoretical is the initial vale according to the manufacturer. Total content of fluoride ions (%) compared with initial values according to the manufacturer are shown.

Table 3

Fluoride cont.ent (%) of the various commercially available formulations A–E and gel

Fomulation	Amount (g)	% F- theoretical	%F-measured (actual)	Total content % F-
A	0.5	125	48.3 ± 3.8	38.64 ± 3.8
B	0.5	140	53.7 ± 4.1	38.35 ± 4.1
C	0.5	130	48.4 ± 1.5	37.23 ± 1.5
D	0.5	120	44.8 ± 2.6	37.33 ± 2.6
E	0.5	136	51.0 ± 2.1	37.50 ± 2.1
Gel	0.3	1250	1105 ± 0.9	88.40 ± 0.9

Content of 10.0 ml solution: quantity 0.5 g, 1.0 ml TISAB®, 10.0 ml distilled water; temperature 30°C, constant stirring speed, $n = 3$, time interval of 3 min, ±2.5% SD, measured with membrane Nadir® in the measuring cell (liberation measurement) used for the simulation of diffusibility in the mouth mucosa. Percentage F-theoretical is the initial value according to the manufacturer. Total content of fluoride ions (%) compared with initial values according to the manufacturer are shown.

on insoluble metaphosphates (preparation A), revealed no noticeable loss of available fluoride ions (Tables 1 and 2).

On average, values of approximately 10% lower resulted for preparations A, B and E than would be expected according to the calculation. It must be noted here that under the in vitro cleaning conditions, measurement was only carried out for 3 min in order to approximate the general time frame in vivo.

Preparations C and D show a loss of available fluoride ions over this period. Sodium monofluorophosphate (MF) combined with calcium carbonate (C) and sodium fluoride combined with calcium hydrogen phosphate dihydrate (D) are the abrasive compounds here. Compared with the starting value of 100% fluoride, the combinations lead to a 25% reduction in the content of available fluoride in case C and an almost 50% reduction in case D in vitro (Table 2). When the measurements were carried out using the cellulose hydrate membrane for the task of which should be seen as its simulation of the diffusion ratio in the mucous membrane of the mouth, the percentage fluoride actual values for preparations A–E were up to approximately 50% less in fluoride concentrations (see Table 3), than for the measurement without a membrane (Table 2).

4. Discussion

The investigation of the liberation (see Table 2) and thus the release rate of fluoride ions in five different commercial preparations of toothpastes clearly illustrates the great importance of the selection of auxiliaries, in this case abrasive substances, for these preparations. Two of the preparations tested, those using calcium carbonate and calcium hydrogen phosphate dihydrate as abrasive compounds, show a decrease in the content of available fluoride ions in vitro (Table 2).

In the case of MFP (C), the active ingredient most com-

monly used today for caries prophylaxis, a reaction with calcium carbonate, apparently occurs to a much lesser extent (ca. 25%) than with sodium fluoride to form calcium MFP, which remains soluble and can release reactive fluoride ions into the enamel [10].

In addition MFP can be hydrolysed in vivo by phosphatases present in saliva and plaque, which increases the concentration of free fluoride ions [11]. In this way, the in vitro loss determined here could be compensated for under in vivo conditions. Where NaF is used (D) with calcium phosphate as the abrasive compound, it must be assumed that calcium fluoride is formed, which is not readily soluble, and that the toothpaste thus loses its caries-protective effect.

The choice of aluminium oxide dihydrate with sodium fluoride (E; Table 2) can be regarded as an efficient active ingredient and auxiliary combination with high activity of fluoride ions. The combination shows only a 12% loss of fluoride in vitro (Table 2) and has the additional practical advantage of aluminium ions which can be intercalated in the appetite in vivo [12]. With a loss of 11%, the gel tested (with no abrasive compound content) can also be considered as stable. The two samples A and B. which use organic fluorides with insoluble metaphosphate (A) and silica (B), likewise show only a very low loss of available fluoride, and are thus an effective combination of fluoride-containing toothpastes for prophylaxis.

An abandonment of the combination of sodium fluoride, tin fluoride, calcium fluoride, potassium fluoride with calcium carbonate or calcium hydrogen phosphate dihydrate in toothpastes would be regarded as realistic. Forsman and Ericsson [1] and Naujoks [19] come to the same conclusion in their investigations. One explanation for the results for measurements performed with the membrane (see Table 3) could be that membrane models such as the diffusion cell (according to Dibbern) employed in the investigation often do not show adequate mixing of solutions, and must therefore be considered as problematic due to the diffusion layers which inevitably build up on the membranes.

As it can be assumed that no one cleans their teeth for longer than 3 min in vivo with toothpaste containing fluoride, there are probably no significant amounts of fluoride absorbed into the mucous membranes of the mouth, and the caries-protective effect of such compounds is a purely local nature, and there is therefore no danger of overdosing and systemic intoxication [13,18].

There must also first be an initial increase in the level of fluoride ions in the saliva, which then covers the oral mucous membrane, but is also partially swallowed [16]. McCall et al. [14] and Einwag and Naujoks [17] also come to the same conclusions. In vitro/in vivo correlation problems must also be noted, i.e. under in vivo conditions, the secretion and composition of saliva, the enzyme activity, the composition of plaque, as well as the cleaning technique and age create additional dispersion criteria which have not been considered in this in vitro investigation.

Only in special cases can conclusions which have a direct relationship to the action be drawn from liberation experiments alone. The requirement for this is that the therapeutic action of the preparation be determined mainly by the active ingredient [15]. This is the case for toothpastes with respect to their effectiveness for protection against caries. This aspect becomes increasingly important when considering the fact, that the addition of fluorides to toothpastes is subject to compliance with the Cosmetics Directive [8] (January 1st, 1997) which states that all auxiliaries have to be declared on products.

If it is assumed that the average content of cleaning compounds in toothpastes is between 20 and 60%, and calcium carbonate is one of the most frequently employed cleaning compounds, it is to be feared that a large number of the fluoride-containing preparations of this composition contain only a fraction of the original F^- concentration as an available constituent, in combination with NaF or SnF_2. If, on the other hand, an additional decrease in content after relatively long storage times and after temperature variations is also taken into consideration, the results of such formulations are very unsatisfactory. The new Cosmetics Decree of January 1st, 1997, which includes the duty to declare all auxiliaries, will in this case probably be advantageous to the transparency, and therefore to the effectiveness of fluoride-containing preparations in prophylaxis.

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