DOES DENTAL FLUORIDE USE HAVE CLINICALLY SIGNIFICANT EFFECTS ON ORAL BACTERIA?

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SUMMARY: Dental research conferences in 1990 and 2001 concluded that fluoride (F) does not produce clinically relevant changes in the number or concentration of bacteria species found in dental plaque. *In vivo* evidence with the F concentrations commonly used in F toothpastes (500–1500 ppm), followed by mouthrinsing with water, has failed to show any clinically significant effect on the bacteria involved in cariogenic activity. Two plausible explanations are possible: (a) various strains of cariogenic oral bacteria have become resistant to F; (b) F never actually had any significant impact on caries-promoting oral bacteria at clinically relevant concentrations. If the first scenario is true, a more resistant cariogenic oral bacteria may have been induced in modern man with the extensive use of F, just as overuse of penicillin and other antibiotics has resulted in resistance to disease-causing bacteria. If the second scenario is true, then various agencies and organizations promoting F use for caries prevention need to re-evaluate their positions based on findings of current dental research.

Keywords: Cariogenic bacteria; Dental caries; Fermentable carbohydrates; Fluoride resistance; Oral bacteria; Oral biofilm; Plaque.

INTRODUCTION: ECOLOGICAL PLAQUE HYPOTHESIS

Unlike the classical model of infections, wherein a single microbe species is found to cause a specific disease, oral pathologies such as dental caries have multiple origins. A new paradigm based on ecological principles appears to be leading to a more effective understanding of the enamel biofilm conventionally called “dental plaque,” which contains more than 500 bacterial species, and displays a high degree of structural and functional organization.[1-6] This new ecological approach forms the basis for evaluating the relationship between fluoride (F) and dental pathology.

With the exception of a limited number of pathogens, the majority of indigenous oral microorganisms are benign or beneficial.[1,4] “Dental plaque forms naturally on teeth and is of benefit to the host by helping to prevent colonization by exogenous, and often pathogenic, species.”[4] Plaque also is a reservoir of key tooth minerals such as calcium and phosphorus, thus enhancing the duration of the remineralization period. It is now known that disruption of the homeostasis in the dental plaque ecosystem is due to changes in influencing factors, such as nutrition, the amount, type, and frequency of fermentable carbohydrates in the diet, immune defense of the host, salivary flow, and oral hygiene (use of antimicrobials and mechanical removal of sugar residues and bacteria).[4,5] Interactions of genetic, socio-economic, nutritional, and even political factors are additional factors. These disruptions lead to changes in the proportions, numbers, and interactions of acidogenic (acid-producing) and aciduric (acid-tolerant) bacteria that are at the core of dental caries activity.[4-6] In the development of caries, bacteria species

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associated with pathological outcomes are increased, while species that normally prevail in good health are reduced.

A decrease in dental plaque pH triggered by the ingestion of fermentable carbohydrates is a "necessary cause" in the increased solubility of calcium hydroxyapatite in the dental hard tissues, leading to a loss of calcium, phosphorus and hydroxyapatite from the tooth surface.[2] Chronically low pH shifts the dynamic balance between demineralization and remineralization in favor of demineralization.[2,4-10] The Stephan curve shows a critical level for demineralization at a pH of approximately 5.5, although the critical pH level varies significantly between low caries groups and high caries groups, demonstrating that factors other than pH are also important.[9]

This multifactorial nature of dental caries makes it evident that simplistic explanations based on mediation of oral pathologies to F use are not valid. The present review critically examines currently available research relating to the clinical significance of the effects on oral bacteria of F in toothpastes, mouthwashes, and topical applications. By extension, the effects of lower levels of F in drinking water, beverages, and foods, on these microbiota are also scrutinized.

FLUORIDE IN THE ORAL CAVITY

At a dental conference in 1990, a participant reported: "To date, studies indicate that fluoride at prophylactic levels in the environment does not influence the composition of dental plaque."[11] Another researcher at this conference observed that after a single application of topical F to plaque, "The effects of [the] treatments were only of short duration and may not be relevant to caries prevention *in vivo*."[12] At a 1992 symposium it was stated that, "Fluoride can also inhibit bacterial growth, but usually at concentrations much higher than those found in dental plaque. The anticaries properties of fluoride, therefore, have not generally been considered to involve its antimicrobial activity."[4] A consensus statement from another dental conference in 2001 stated that there was "sparse evidence of the possibilities of antimicrobial caries prevention" with the use of topical F.[13]

Earlier views that F needs to be ingested (systemic use) for the prevention of dental caries, by changing the tooth structure, have been refuted by newer findings.[14] Many sources of systemic F exposure are simultaneously available, including F dental products, which can rapidly deliver systemic F, owing to its quick uptake in the richly vascularized tissues of the mouth, and to its accidental ingestion; F in foods due in part to the widespread use of phosphate fertilizers, which often contain appreciable amounts of F; as well as F-containing pesticides and post-harvest fumigants such as sulphuryl F; and even F in air pollution. In France, systemic F is officially restricted to only one source in an effort to control F intake and minimize harm to health.[15] The most significant study that has examined caries experience in relation to individual F intakes at various ages during childhood (the Iowa F study) found no association between F intake and caries experience.[16] One study which examined how the removal of one source of

systemic F (fluoridated water) affected oral bacteria, found no significant change in either the F concentration in plaque or the level of *S. mutans* in the biofilm.[17]

SALIVA FLUORIDE

Salivary flow is one of the many factors influencing caries because of the ability of this biological compartment to bathe the teeth and the oral biofilm.[18] When fluoridated water is ingested and the F is excreted back out through the salivary ducts, F levels in saliva barely increase above baseline.[19,20] A comparison of F uptake from water and dentifrice showed that a F concentration of 0.85 mg/L in drinking water barely elevates salivary F.[19] The consumption of fluoridated water leads to a F concentration of about 0.02 ppm in resting saliva, compared to 0.01 ppm that is normally the baseline.[21] According to the US Centers for Disease Control and Prevention, "This concentration of fluoride is not likely to affect cariogenic activity."[21]

When 1000-ppm F toothpaste is used, F saliva levels start at 1000 ppm (as soon as the dentifrice touches the teeth) and then become diluted as saliva mixes with the toothpaste. After brushing for approximately 2 min, F saliva levels are diluted further. If not all the toothpaste is held in the mouth for 2 min, F saliva levels decrease even more. After expectoration, F saliva levels are as low as fluoridated drinking water (1 ppm).[22] Rinsing with water after toothbrushing also reduces F in saliva from topical F toothpaste to clinically insignificant values.[23-28] "Post-brushing water rinsing may reduce the risk of fluoride ingestion from dentifrice, however the decreased salivary fluoride bioavailability may compromise any consequent anticaries benefits."[28]

Fluoridated toothpicks delivered approximately the same F concentration in saliva as the proximal brush dipped in 905 mg/L F gel or rinse, but the F concentrations remained elevated for 1 hr.[29] A study using fluoridated salt[30] showed that salivary F concentrations peaked 1 or 2 min after eating but returned to baseline after 20 min.

PLAQUE COMPOSITION

A number of studies have examined the effects of topical F applications on plaque and bacterial composition. F levels in plaque after brushing with 1000-ppm F toothpaste returned to baseline values after 30 min.[18,31] In earlier work, bacterial recovery in plaque was apparent after only 30 min following a single exposure to F.[32] When plaque and saliva composition were analysed after a F rinse and subsequent sucrose application, no correlation between F levels in plaque and formation of lactic acid was found.[33] A comparison of the concentration of acid-soluble F ions in the plaque of caries-free and high caries groups of subjects found no significant differences.[34] A study using 1500 ppm F in a toothpaste was found to be insufficient to interfere with bacterial growth and metabolism.[35] In a cross-over study, researchers found that "fluoride varnish with or without preceding dental prophylaxis had no significant effect on the plaque and salivary levels of *S. mutans*."[36]

At this point, the content and complex interactions of the various components of plaque are not completely understood, making any simplistic conclusions regarding effects of F based on *in vitro* tests unreliable for several reasons. Firstly, in a plaque ecosystem where interactions of the various components in the ecosystem are important, *in vitro* research results do not accurately reflect the complexity of *in vivo* events because the experimental techniques actually disrupt the integrity and function of this ecosystem.[37] **"**Organization of micro-organisms within biofilms confers, on the component species, properties that are not evident with the individual species grown independently or as planktonic populations in liquid media."[38] Secondly, although *in vitro* experiments have demonstrated an impact of F on some pathogenic bacteria such as *S. mutans*, other pathogenic bacteria such as lactobacillus and actinomyces species are resistant to any effects (see below). *In vitro* studies that selectively omit bacteria such as lactobacillus, which is known to be resistant to F, in an attempt to induce an antibacterial effect, cannot be said to be clinically relevant.[7]

Recent research has tested the hypothesis, in a double-blind, crossover clinical study, that NaF in a toothpaste exerts a demonstrable effect on the composition of dental plaque, and on the bacterial component in particular.[39] It was found that the pathogenic bacteria numbers and proportions associated with caries were not significantly reduced in plaque between teeth (interproximal surfaces) after using F toothpaste.[39]

Clinically significant effects of any agent used to protect teeth requires the levels be high enough in the site of action within plaque.[40] "Estimates of fluoride concentrations required to inhibit bacterial acidogenesis vary from 10 mg/L to 1900 mg/L."[40] However, this research failed to measure the extent to which F penetrates the plaque biofilm to the enamel tooth surface where demineralization occurs. The degree to which F penetrates plaque is related to the concentration of F, the duration of F exposure, and other factors.[40] Although *in vitro* investigations demonstrate the potential for F to penetrate the biofilm if F concentrations are high enough, and if sufficient time is allowed, such investigations have little relevance to the average "tooth-brushing" event. The first researchers to examine the penetration of F toothpaste (NaF at 1000 ppm F) into natural plaque biofilms generated *in vivo*, on natural enamel surfaces, for 30 or 120 sec, deemed to be the equivalent to normal toothbrushing behaviour, found the plaque F concentrations that are inhibitory of acidogenesis were "attained only in the outermost plaque layers and decrease when exogenous NaF is removed."[40] The F concentrations near the enamel surface, where demineralization occurs, were low, leaving many bacteria unexposed to F.[40]

Research has therefore failed to demonstrate that clinical applications of F significantly affect oral bacterial. If high F concentrations, used directly on the teeth are unable to control microbial activity, it seems unlikely that the low F concentrations in fluoridated drinking water can limit microbial growth or metabolism in any clinically relevant manner.

BACTERIAL RESISTANCE TO FLUORIDE

Chlorhexidine and iodides are broad-spectrum antimicrobials. It is now generally accepted that "fluoride is not generally bactericidal."[8] Many bacterial strains are resistant to F, including: lactobacilli, actinomyces spp, and some streptococci.[6,41] No bactericidal activity of F against *S. mutans* aggregates, even at the saturated concentration of 34,000 mg/L at pH 3.5, were reported.[41] A study comparing chlorhexidine and F toothpastes showed that only chlorhexidine showed significant efficacy against oral bacteria 1 to 6 hr after use.[42] The use of iodide constitutes "a valuable adjunct to current periodontal therapy because of its broad-spectrum antimicrobial activity, low potential for developing resistance and adverse reactions, wide availability, ease of use, and low financial cost."[43]

Human dental plaque may be colonized by F-resistant bacterial strains in response to F pressure.[8,12] This exposure to F may provide an ecological advantage to F-resistant bacteria to colonize the dental plaque. Although it has been postulated to occur under normal physiological conditions, this is unlikely.[7] What is unknown, is whether the bacteria in our mouths have always been resistant to F or whether they have adapted to the ubiquitous presence of F. After countless generations of bacteria whose ancestors might have initially been affected by F, the question remains as to whether these same bacteria have now adapted to ubiquitous low levels of F.

HEALTH RISKS FROM DENTAL FLUORIDE

A current hypothesis suggests that high concentration topical F applications may have an impact on caries through the formation of calcium fluoride (CaF_2) stored locally in plaque and on the surface enamel, which acts as a short-lived reservoir of substrates for remineralization.[44] As a result, many researchers are now recommending 5000 ppm F in toothpaste.[45-49] From a comprehensive analysis by Akiniwa, these increased F concentrations definitely increase the possibility of acute or chronic F toxicity to the general population.[50] Several case reports have been published demonstrating chronic F toxicity from the use of F toothpaste.[51] Flavoring toothpastes to make them taste "yummy" may also encourage individuals, especially children, to swallow F toothpastes. It is well-known that young children have not fully developed the reflex to expectorate.[52] This developmental limitation increases the likelihood that children will inadvertently swallow F toothpaste.

F ions are known to interfere with many enzymes throughout the body, therefore it was hypothesized that F anti-microbial action involves the direct inhibition of enzymes in oral bacteria.[8] One enzyme system that helps micro-organisms survive the acid they produce during the consumption of fermentable carbohydrates is the arginine deiminase enzyme system (ADS). The ADS involves generation of ammonia and carbon dioxide which effectively neutralize acids.[8,53,54] Although earlier research[53] seemed to indicate that F suppresses the ADS system, more recent research by the same team demonstrated that NaF was a poor inhibitor of the ADS of oral bacteria using clinically relevant F concentrations.[54]

When combined with aluminum, F is a potent inhibitor of another enzyme called ATPase.[55] ATPase enables micro-organisms to protect themselves against acid challenges by maintaining an intracellular pH which is 0.5 to 1 unit higher than the extracellular environment.[8] It has been suggested that this F mechanism is important in the protection of acidogenic bacteria such as lactobacilli,[8] yet lactobacilli are known to be resistant to F.[2,6,7,41]

In teeth, the low pH environments created by fermentable carbohydrates cause aciduric and acidogenic bacteria to take up F as hydrogen fluoride (HF), which dissociates intracellularly, acidifying the cytoplasm.[8] Recent evidence demonstrates that these weak-acid effects by F also create a more toxic environment for ameloblasts by interfering with various pathways and molecular processes.[56]A low pH environment also facilitates the entry of more F into these tooth cells, causing increased cell stress that compromises ameloblast function and viability.[56] Of concern is whether F similarly causes cell stress in bone (osteoblasts and osteoclasts) or in other F-accumulating tissues. Other research found that F entry into ameloblasts and osteoblasts adversely affects crystal formation in tooth and bone respectively at all levels of F intake.[57]

ALTERNATIVE PERSPECTIVES

Poor nutrition, coupled with frequent, daily consumption of processed carbohydrates, results in high rates of oral pathologies, including tooth decay, as well as the malformation of the dental arch and craniofacial development.[58-61] In the USA, 1% of children under 5 years old have frank malnutrition, 10% of households report food insecurity, and 3.3–21% of children are underweight and stunted due to inadequate nutrition for various US regions and populations.[61] Primitive cultures which had adequate nutrition, with minimal exposure to fermentable carbohydrates, had negligible oral diseases, without the use of toothbrushes, toothpaste, or specialized dental care, such as the removal of dental plaque.[58,61]

A recent publication provides an extensive list of traditional medicinal plant extracts and natural products with activity against oral bacteria.[3] Two recent reviews consider various non-invasive, calcium-phosphate-based oral delivery systems.[62,63] Maintenance of saliva supersaturated in calcium and phosphate provides the substrate needed for dental remineralization. Many of these products are safe to ingest, unlike F, which poses a safety risk if significant amounts are swallowed.[64]

It has been known for over 40 years that the use of sugar-free chewing gum is effective in the prevention of caries due to the stimulation of salivary flow.[65,66] The results of six controlled clinical studies have indicated that chewing such sugar-free gum after meals results in a significant reduction in the formation of dental caries.[66] The increased rate of saliva increases the concentration of bicarbonate which neutralizes the acids, leading to an increase in plaque pH that favors remineralization of the tooth.[61] Additional evidence also indicates that carbohydrate alcohols such as xylitol and sorbitol have significant noncariogenic and even anticariogenic properties.[67]

CONCLUSIONS

Although recent epidemiological studies periodically report that F intake via water fluoridation helps prevent dental caries, these claims remain unconvincing, primarily due to the failure to control for the more than 100 factors known to influence caries,[68] including the ability of F to delay the eruption of teeth.[69] Delay in the eruption of teeth decreases the exposure time to cariogenic conditions, thus confounding any age-specific analyses of caries rates. For epidemiological evidence to be convincing, there must be adequate control for the many variables that influence caries.

Although *in vitro* studies suggest that F may have subtle anti-microbial effects, the *in vivo* evidence using F concentrations commonly used in toothpastes (500–1500 ppm), with subsequent mouth-rinsing with water, fails to demonstrate any clinically significant antagonistic effect on the bacteria involved in cariogenic activity. In fact, no available research has shown that F at 1 ppm in water significantly alters plaque metabolism or plaque growth (bactericidal effects).

To this reviewer, two plausible explanations are possible: a) the various oral bacteria have become resistant to F; b) F never had any significant impact on oral bacteria. If oral bacteria are becoming more resistant to F, a more cariogenic flora has been induced in modern man with the use of F, just as overuse of antibiotics has resulted in bacteria becoming resistant to many of them, including penicillin. Therefore, if F never actually had any significant impact on oral bacteria, then F-promoting agencies and organizations clearly must re-examine the best available dental research using the ecological paradigm as a basis for the evaluation of any relationship between F and dental pathology.

At a minimum, future dental research will need to address the follow questions. Firstly, if it is accepted that disease is defined as a destructive process with a specific etiology, is dental caries, which is caused by multiple and complex influencing factors, best characterized as a disease? According to one researcher, “Caries lesions are often confused for the disease process” and “fluoride may have little or no effect on the disease process per se.”[9] Secondly, what is the relative efficacy of F products in optimizing plaque ecosystems for oral health when compared with other proposed modalities such as sugarless gum, and calcium-phosphate delivery systems? Thirdly, what role has (a) the improvement in dental and health education, (b) an improved standard of living which improved nutrition for the growing middle class, (c) the introduction of dental floss and dental sealants, (d) campaigns to promote breast feeding to counter corporate promotion of infant formula, which impacts the prevalence of baby bottle tooth decay, (d) the availability of sugar substitutes, and (e) the educational attempts to teach citizens that a third set of teeth (i.e., dentures) is not inevitable,[58] had on caries prevention, to compensate for the increased use of cariogenic food items such as sugar and white flour? Finally, do the benefits of high concentration topical F in dental products outweigh the risks of acute and chronic toxicity?[50,51]

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