The biological plausibility for the caries preventive action of fluoride in milk

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Introduction

Biological plausibility is an essential stage in establishing the therapeutic value of a preventive or curative agent. Ultimately, efficacy and effectiveness are tested in clinical trials and community programme evaluations, which provide reassurance and guidance for potential users. Almost invariably, biological plausibility is demonstrated before clinical evaluations are sanctioned and undertaken. Biological plausibility can be established through a variety of investigations relevant to the disease or disability. As far as dental caries is concerned, the biological process is so well known so that there is little difficulty in accepting a variety of models which may be used to demonstrate biological plausibility. These models include systemic parameters (eg. plasma and urine) and intra-oral parameters (eg. saliva, plaque and enamel). This applies particularly strongly to the preventive agent fluoride since its biological effects have been studied very extensively.

The ability of fluoride to prevent dental caries was first observed when present as a natural constituent of drinking water. Since then, fluoride has been incorporated into a wide variety of vehicles so as to satisfy different clinical situations and needs. These vehicles include milk, salt, dietary supplements, toothpastes, mouthrinses, gels, varnishes and glasses. The concentrations of fluoride in these vary greatly, as some were developed for topical use only and others for systemic use, some for infrequent use and others for frequent use, and some for individual patients and others for communities. The means by which fluoride prevents caries for each of the above agents is mostly well established, although mechanisms will differ in relative importance between these disparate methods. For groups of agents, the mechanisms by which fluoride prevents caries is likely to be similar – for example, gels and varnishes, which are applied to teeth infrequently with fluoride present in very high concentration. Milk and water have much in common as vehicles for fluoride, in that the concentration of fluoride is low and they are both drunk as part of the diet. The clinical effectiveness of fluoride in water is well established (WHO, 1994; NHS CRD, 2000; MRC, 2002). The next section will examine the extent to which fluoride in milk and fluoride in water share caries preventive mechanisms and the extent to which these mechanisms demonstrate biological plausibility.

Caries preventive mechanisms of fluoride in milk and fluoride in water.

Plasma fluoride profiles.

Direct comparisons of the plasma fluoride profiles in fasting human subjects following ingestion of fluoride in either water or milk have shown that absorption from milk is initially much slower than from water, but the increase in plasma fluoride is more prolonged from milk with the result that the cumulative uptake is approximately 70-75% compared with water (Ekstrand and Ehrnebo, 1979; Spak, Ekstrand and Zylberstein, 1982; Trautner and Siebert, 1986). The reduction in bioavailability by milk is believed to be the result of physical entrapment of fluoride by coagulated milk proteins.

However, the cumulative uptake of fluoride from milk, when taken with food, is similar to that from water in fasting subjects (Trautner, 1989; Trautner and Einwag, 1989). The plasma fluoride peak is lowered, but prolonged considerably by the presence of food; it is thought that food delays gastric emptying, thus allowing digestion of milk proteins responsible for entrapping fluoride.

Studies in animals confirm the reduction in plasma uptake of fluoride in milk, compared with water, when fasting (Ericsson, 1958; Patz, Henschler and Fickenscher, 1977), but the effect of concomitant food intake on plasma fluoride levels appears not to have been studied directly in animals. However, Villa et al. (1989), studying bone fluoride uptake in rats, found that with *ad libitum* access to food, fluoride uptake was higher from sodium monofluorophosphate in milk than from sodium fluoride in water, but that when fluids and solids were administered separately, uptake was similar from both fluoride sources, indicating facilitation of fluoride absorption by foods.

To summarise, systemic bioavailability of fluoride is reduced by around 25% when in milk compared with water under fasting conditions, but this effect is abolished if stomach emptying is delayed, allowing complete fluoride absorption.

Urinary fluoride excretion.

Ericsson (1958) showed that, in fasting human subjects, the urinary excretion of fluoride following the consumption of fluoridated milk was delayed, and the overall excretion was about 80% of that from water, consistent with the conclusions from plasma fluoride profiles. Similar conclusions were drawn from the results of Ekstrand and Ehrnebo (1979), Trautner and Siebert (1986), Shulman and Vallejo (1990) and Brambilla et al (1995).

Marthaler et al (1978) proposed that systemic fluoride supplementation schemes should use urinary fluoride excretion measurements to adjust the fluoride dose to achieve urinary outputs equivalent to that found in comparable subjects from water fluoridation areas. This proposition assumes that the mode of action of fluoride from all fluoridation schemes is similar. The urinary fluoride excretion in 4-6 year old children living in optimally fluoridated areas is around 0.4 mg fluoride per day (Rugg-Gunn et al., 1993; Baez, Baez and Marthaler, 2000). For children of similar age receiving fluoridated milk (most frequently, approximately 1 mg of fluoride per day), urinary fluoride excretion is close to 0.4 mg per day (Villa et al., 1989; Kolesnik, 1997).

With water and salt fluoridation, the fluoride intake is divided into several small doses through the day, while with milk the normal mode of administration is in a single daily dose. It is of interest that the fractional excretion of fluoride from small doses is higher than that from a larger dose (Ketley and Lennon, 2001; Villa et al, 2000). Thus, normalising urinary fluoride outputs on water fluoridation data could lead to a slightly greater fluoride intake from milk than from water.

In summary, urinary excretion data confirm the reduced bioavailability of fluoride from milk compared with water in fasting conditions. The most frequently used dose of fluoride in milk (1mg/day) leads to a urinary fluoride output comparable to that from similar children consuming optimally fluoridated water.

Uptake of fluoride by hard tissues; animal experiments.

König (1960) found that uptake of fluoride into the skeleton (and by implication, into the developing teeth) of weanling rats was higher from fluoridated milk than fluoridated water, when given to the dams during pregnancy and lactation. However, no inhibition of caries was observed when the pups were subsequently fed a cariogenic diet: only when the pups were given fluoride simultaneously with the cariogenic diet was caries inhibited. This finding was one of the first experiments showing the importance of post-eruptive exposure to fluoride in caries prevention. Similar caries results were found by Poulsen, Larsen and Larson (1976); no significant differences in caries development were observed compared with fluoride-free controls when the rats were given fluoride in milk or water by stomach tube before tooth eruption from day 5-15 after birth, but caries was reduced when the animals were given fluoride in 3 ml of milk or water once a day together with cariogenic diet, after weaning at about 21 days. Fluoride uptake into the enamel was higher from milk than from water with both pre- and post-eruptive administration.

Schori et al. (1976), measuring fluoride uptake into the molars and femurs of rats receiving various fluids containing fluoride all at 2.9 ppm, found that supplementation of tea, milk, and tea with milk resulted in similar levels of bone fluoride, but that fluoride-supplemented tea alone gave higher fluoride uptake into molar enamel than fluoride-containing milk or tea with milk. However, Cutress et al. (1996), in a study of the incorporation of fluoride into developing ovine incisors from milk or water, found that fluoride uptake into the teeth was independent of the vehicle.

In summary, the effect of milk on pre- and post-eruptive fluoride uptake into hard tissues in animals is equivocal; uptake is increased, reduced or equal to water in different studies.

Uptake of fluoride by tooth tissues; in vitro and in vivo studies in man.

Fluoride concentrations in the incisor enamel of 8-10 year old children who had received fluoride-containing milk for the previous 5 years (i.e. before and after eruption of the permanent incisors) were increased compared with controls not receiving fluoride supplementation (Tóth et al., 1987). This increase in fluoride levels was accompanied by a non-significant reduction in enamel solubility. The study was part of a clinical trial of milk fluoridation that demonstrated a 60% reduction in caries development. A 3-year trial of milk fluoridation in children initially 4-7 years old (Zahlaka et al., 1987) resulted in a significant reduction in caries in both deciduous and permanent teeth, but no difference in enamel fluoride content, contrary to the findings of Tóth et al. (1987). A longitudinal study of enamel fluoride uptake by Tóth et al. (1989), in which children were given 1 mg fluoride in milk for 12 months and enamel biopsies carried out before and after 6 and 12 months, showed a significant reduction in enamel solubility (measured as phosphate content of the biopsy sample) at 6 and 12 months, and an increase in fluoride at 12 months compared with baseline.
An *in vitro* study by Tóth et al. (1997) of the uptake of fluoride into surface enamel and anti-solubility effects of exposure of demineralised enamel for 7 or 14 days to milk containing 0, 1, and 10 ppm fluoride, showed significant increases in enamel fluoride concentration, and reductions in acid solubility, only with 10 ppm fluoride after 14 days. Rugg-Gunn and Boteva (1997) compared enamel fluoride uptake *in*

vivo when enamel samples were mounted in an intra-oral device and exposed 4 times daily to fluoridated milk or water. The enamel samples were either sound or experimentally demineralised, and the fluoride supplements were either rinsed alone, or rinsed and then ingested. For both sound and demineralised enamel, fluoride uptake was greater with milk than with water, and greater when the milk or water was rinsed and ingested compared with rinsed alone.

To summarise, fluoride is taken up by human tooth tissues from fluoridated milk both pre- and post-eruptively. When compared with water in this respect, there is evidence that milk is a more effective vehicle for fluoride delivery than water.

Fluoride in saliva and plaque.

Twetman, Nederfors and Petersson (1998) measured fluoride levels in whole saliva, parotid and submandibular saliva from school children before and after 7 days consumption of fluoridated milk (1 mg daily). The salivary fluoride levels were significantly elevated in whole saliva at 1 and 3 hours, and in duct saliva up to 6 hours, after milk ingestion, compared with baseline. Gintner, Phillips and Bánóczy (2000) measured fluoride in stimulated whole saliva before and after rinsing with water or milk containing 5 ppm fluoride; similar fluoride peaks were found with both vehicles 5 minutes after rinsing but, while the fluoride level fell with water, the increased fluoride could still be detected after 2 hours with milk. However, Rugg-Gunn and Boteva (2000) found that more fluoride could be found in unstimulated saliva collected for 4 minutes after rinsing with fluoride milk compared with water, but that with both vehicles, the fluoride concentration fell in the second 4-minute samples to near baseline values, with no difference between milk and water. Boros, Keszler and Bánóczy (2001) showed increases in the salivary and urinary fluoride concentrations when 200 ml of fluoridated milk (5 ppm) was ingested, but not when the milk was rinsed only. Labial gland saliva contained about 10 times the fluoride level of unstimulated whole saliva, and did not change after fluoridated milk ingestion.

Petersson et al. (2002) found elevated fluoride levels in unstimulated saliva at 15 (but not 120) minutes after ingestion of 200 ml of water or milk containing 5 ppm fluoride. Plaque fluoride levels were, however, higher with fluoride-containing water and milk after 120 minutes, compared with baseline or fluoride-free water and milk. Engström (2002) showed that plaque fluoride levels were raised for up to 4 hours after fluoride milk ingestion. Kertész, Gombik and Bánóczy (1992) found that after 8 weeks consumption of milk containing 2.5 ppm fluoride, plaque fluoride was raised, and the numbers of mutans streptococci in plaque fell; in controls, the mutans count rose. Engström et al. (2004a) found no significant changes in salivary microflora after daily intakes of fluoridated milk, but the same group (Engström et al., 2004b) showed that lactic acid formation in sucrose suspensions of plaque collected 30 minutes after drinking fluoride-free milk rose, but not after fluoridated milk.

In summary, fluoride concentrations in saliva and plaque rise after intake of fluoridated milk, as occurs with fluoridated water. There is some evidence that the retention in plaque and saliva of fluoride from milk is greater than that from water. Ingested fluoride may be re-secreted in saliva.

Animal caries studies.

As described above, König (1960) and Poulsen, Larsen and Larson (1976) showed that both fluoridated milk and fluoridated water reduced caries in rats when administered at the same time as the cariogenic diet, but not pre-eruptively. Bánóczy et al. (1990) found that fluoridated milk was more effective in reducing rat caries than fluoride in water: milk alone reduced caries compared with water-only controls, consistent with data from König (1960) above, and Shaw, Ensfield and Wollman (1959). The caries-reducing effect of milk was observed by Stösser, Kneist and Grosser (1995), who also demonstrated caries reductions with milk supplemented with sodium fluoride, sodium monofluorophosphate and sodium hexafluorosilicate compared with milk alone or water controls.

The animal caries results suggest that fluoridated milk is as, or in some studies more, effective in reducing caries than similar concentrations in water.

De- and remineralisation in vitro and in vivo.

As mentioned above, Tóth and colleagues observed reduced solubility in enamel in children receiving fluoridated milk compared with baseline (Tóth et al., 1989), and *in vitro* after exposure to 10 ppm fluoride in milk for 14 days (Tóth et al., 1997). Al Khateeb et al (1998) showed increased *in vitro* remineralisation of artificial lesions in human enamel by exposure to fluoridated milk, but no difference in effect between three fluoride levels (1, 2.5 and 5 ppm) was found. Rugg-Gunn and Boteva (2000) reported increased *in vitro* remineralisation of white spot lesions with fluoridated milk compared with fluoride in water or milk alone. A brief report by Wang et al. (2001) indicated that *in situ* remineralisation of lesions in bovine enamel was enhanced by fluoride in milk compared with fluoride in water.

An *in vitro* pH cycling model was used to study the effect of 1 ppm fluoride in milk on demineralisation of enamel over 99 days (Arnold et al., 2003). The effects were measured by serial sectioning followed by computerised reconstruction of the lesions. From the results, the authors concluded that fluoridated milk prevents *in vitro* demineralisation in three ways: fluoride binds to calcium to form a reservoir of loosely bound fluoride; calcium and phosphate from milk contribute to remineralisation, and milk proteins are adsorbed onto the enamel surface and reduce demineralisation. Another *in vitro* pH cycling study of the effect of fluoride in milk on root surface lesion progression (Ivancacova et al., 2003), indicated a reduction in the rate of lesion progression by milk alone, and by fluoridated milk, compared with a water control.

These results suggest that fluoridated milk may be a more effective way of administering fluoride than water, either because of increased retention of fluoride from milk, or because of the caries-preventive effect of the milk *per se*.

Discussion

The general understanding of the mode of action of fluoride in reducing the incidence of dental caries is that an elevation of the concentration of the ion at the plaque-enamel interface results in a reduction in the rate of demineralisation, an increase in the rate of remineralisation, and a reduction in the rate of acid production in plaque. The required elevation in fluoride concentration is small but prolonged; large increases in intra-oral fluoride following use of dentrifices, mouthrinses or tablets are soon cleared from the mouth, but residual reservoirs release fluoride slowly over a period of hours or even days to exert the above effect. Ingested fluoride is also re-secreted in saliva, and fluoride that is incorporated in the crystals of the surface layers of the enamel during tooth formation may be released during an acid attack and re-deposited in the subsurface enamel where the developing carious lesion may be arrested.

The above review of the actions of fluoride in milk and in water demonstrates that ingestion of fluoridated milk increases the concentration of fluoride in saliva, as does the ingestion of fluoridated water. The ingestion of fluoridated milk increases the concentration of fluoride in dental plaque and there is some evidence that it decreases acid production in plaque induced by exposure to sugar, as occurs with the ingestion of fluoridated water. There is some evidence that the ingestion of fluoridated milk increases the concentration of fluoride in enamel, both pre- and post-eruptively, as occurs after ingestion of fluoridated water. Fluoridated milk decreases enamel demineralisation and increases enamel remineralisation *in vitro* (and so far as they have been studied, *in vivo)*, as does fluoridated water. It would appear that, at an appropriate concentration (about 1mgF/L in water and about 5mgF/L in milk), fluoride in milk exhibits similar properties conducive to caries prevention, to fluoride in water. For some of the evidence -- for example, caries animal experiments – these caries-preventive actions may be greater in milk than in water.

Conclusion

The effectiveness of water fluoridation is established (WHO, 1994; NHS CRD, 2000; MRC, 2002). This review has shown that fluoride in milk behaves similarly to fluoride in water in the important actions which are known to aid caries prevention. The biological plausibility of fluoridated milk as a caries preventive measure is established.

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