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ABSTRACT

It has been suggested that systemic fluoride absorption from drinking water may be influenced by the type of fluoride compound in the water and by water hardness. Using a human double-blind cross-over trial, we conducted this study to measure c_{max}, T_{max}, and Area Under the Curve (AUC) for plasma F concentration against time, following the ingestion of naturally fluoridated hard and soft waters, artificially fluoridated hard and soft waters, and a reference water. Mean AUC over 0 to 8 hours was 1330, 1440, 1679, 1566, and 1328 ng $F.min.mL^{-1}$ for naturally fluoridated soft, naturally fluoridated hard, artificially fluoridated soft, artificially fluoridated hard, and reference waters, respectively, with no statistically significant differences among waters for AUC, c_{max}, or T_{max}. Any differences in fluoride bioavailability between drinking waters in which fluoride is present naturally or added artificially, or the waters are hard or soft, were small compared with large within- and between-subject variations in F absorption. Abbreviations used: F, fluoride; AUC, Area under the Curve for plasma F concentration against time; AUC(0-3), Area under the Curve for plasma F concentration against time for 0 to 3 hours following water ingestion; AUC(0-8), Area under the Curve for plasma F concentration against time for 0 to 8 hours following water ingestion; c_{max}, maximum plasma F concentration corrected for baseline plasma F and dose (*i.e.*, F concentration of individual waters); T_{max}, time of c_{max}.

KEY WORDS: bioavailability, drinking water, fluoride, water hardness.

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INTRODUCTION

Fluoride occurs naturally in tap drinking-water supplies in the United Kingdom (UK), at concentrations up to 1.5 milligrams *per* liter. As a means of reducing the prevalence of dental caries, low F concentrations in drinking-water in selected areas of the UK may be increased to a target concentration of 1 milligram *per* liter by the addition of hexafluorosilicic acid or its sodium salt. However, most of the evidence relating to the long-term general health effects of F is based on studies of populations living in areas receiving F occurring naturally in water supplies.

A recent systematic review on the health effects of F, conducted by the University of York Centre for Reviews and Dissemination (McDonagh *et al.*, 2000), concluded that "the assessment of natural versus artificial water fluoridation effects is greatly limited due to lack of studies making a comparison". A subsequent MRC Working Group, established to determine what further research was required to improve knowledge on F and health, identified bioavailability of naturally fluoridated water *vs.* artificially fluoridated water and of hard *vs.* soft water, as important areas of uncertainty (Medical Research Council, 2002). Higher natural levels of F occur typically (but not exclusively) in hard water, while artificial fluoridation usually involves softer water sources. Water hardness is principally determined by the concentrations of calcium and magnesium ions in water and may be expressed as the equivalent concentration of calcium carbonate. Fluoride is present in water supplies as F ions, due to the almost complete dissociation of the parent F compounds, occurring either naturally (predominantly from minerals such as fluorspar [calcium fluoride]) or added as hexafluorosilicic acid or its sodium salt (Department of the Environment, 1987; Her Majesty's Government, 1991). The undissociated proportion is between 10^{-18} and 10^{-30} of the dissociated F ions over the pH range of 6-9 usually found in water supplies, and when the impact of water hardness on dissociation is included, the proportion of free F ion ranges from 91% for water with very high hardness, to more than 99% for soft water (Jackson *et al.*, 2002). It is thought that the absorption of soluble F from the gut into the bloodstream is essentially complete at approximately 95% (Whitford, 1996). However, it has been suggested that absorption may be influenced by the type of F compound in the water and by the water hardness, with the presence of calcium decreasing uptake of F from the gut into the bloodstream, perhaps through ion-pairing, although the importance of any such effect is unclear (Medical Research Council, 2002). A review of the chemistry and bioavailability aspects of F in drinking-water (Jackson *et al.*, 2002), concluded that, "In terms of chemistry and bioavailability there is absolutely no difference between added and 'natural' fluoride", and that, "The effect of major cations—calcium and magnesium (hardness) and sodium—on the chemical speciation and hence bioavailability of fluoride is very small". This review was based on *in vitro* work and a small number of animal studies. With the exception of mineral water (Trautner and Einwag,

1986) and a dilute sodium fluoride solution (Ekstrand *et al.*,1994), there have been no human studies reported on the bioavailability of F from tap drinking-waters.

The aim of this study was to compare the bioavailability of fluoride in natural and artificially fluoridated drinking-waters, and soft and hard drinking waters, using a human experimental model.

MATERIALS & METHODS

Subjects

Ethical approval for the study was obtained from the Newcastle and North Tyneside Local Research Ethics Committee. Thirty-six healthy adults, aged between 20 and 35 yrs, volunteered to take part in the study. Twenty subjects with no history of metabolic disease or acid-base disturbance, and who were not receiving a therapeutic diet, were recruited, having given written informed consent.

The subjects were provided with fluoride-free toothpaste and were asked to avoid using any significant fluoride products one week before, as well as during, the whole six-week experimental period. They were also asked to avoid drinking tea and beer and eating seafood during the washout and experimental periods and were provided with low-fluoride bottled waters (< 0.02 mg F/L) for drinking and cooking.

For the 11 males and nine females who completed all aspects of the study, the mean (± SD) age, weight, height, and body mass index were 25.7 (± 4.5) yrs, 62.7 (± 12.1) kg, 163.1 (± 9.0) cm, and 23.3 (± 2.3) kg/m^2, respectively, for females, and 25.4 (± 2.5) yrs, 75.5 (± 6.2) kg, 175.8 (± 6.3) cm, and 24.5 (± 2.4) kg/m^2 for males.

Experimental Design

The study was a human experimental double-blind cross-over trial, comparing observations within individuals. Over the six-week experimental period, each subject underwent 5 experimental sessions, with a different water consumed on each session and an interval of approximately one week between sessions. Test and reference waters were allocated randomly to the 5 experimental sessions by the project statistician. The test drinking waters were:

- artificially fluoridated soft tap water (AFS) containing 1.01 mg F/L and 50 mg $CaCO_3$/L,
- artificially fluoridated hard tap water (AFH) containing 0.97 mg F/L and 382 mg $CaCO_3$/L,
- naturally fluoridated soft bottled water (NFS) containing 1.06 mg F/L and 63 mg $CaCO_3$/L, and
- naturally fluoridated hard tap water (NFH) containing 0.91 mg F/L and 381 mg $CaCO_3$/L.
- The reference water (REF) was Water for Irrigation BP (British Pharmacopoeia BP) with sodium fluoride added, containing 1.02 mg F/L and 3 mg $CaCO_3$/L (Pharmacy Department, Royal Victoria Infirmary, Newcastle upon Tyne, UK).

All test waters came from UK tap water supplies apart from NFS, which was an Italian mineral water (Acqua Panna, Tione Spring, Italy, bottled by Panna SpA, Italy).

Sample Collection

After an overnight fast, a forearm vein of each subject was cannulated, and a 5-mL blood sample was taken at baseline (T_0). Following the subject's ingestion of 500 mL of test or reference water at room temperature, a further 11 blood samples were collected at regular intervals over the next 8 hrs.

During each session, each subject consumed a specified low-F drink, meal, and snack at 11.00, 12.00, and 15.00 hours, respectively (fluoride content *per* serving was 10 μg F, < 10 μg F, and < 1 μg F, respectively). The same food and drink items were consumed in each of the 5 sessions.

Analytical Procedure

Fluoride concentrations in plasma (ng/mL) were measured by means of a F-ion-selective electrode and the "Known Addition-Slope Determination Technique" (Ekstrand, 1977; Ekstrand *et al.*, 1977). Fluoride concentrations in foods were measured with a F-ion-selective electrode after acid diffusion with hexamethyl-disiloxane (HMDS), as described by Venkateswarlu (1992) and modified by Zohouri and Rugg-Gunn (1999).

The validity and reliability of the methods used in this study have been tested and described elsewhere (Ekstrand, 1977; Zohouri and Rugg-Gunn, 1999). The Coefficient of Variation (CV%) for the "known addition technique" method used was 9.7%. Results of re-analysis of plasma samples were within 0.64 ng F/mL.

Pharmacokinetic Analysis

To determine the increase in plasma fluoride concentration following consumption of the drinking-water, we derived a "baseline-corrected" plasma fluoride concentration "c" by subtracting the baseline (T_0) plasma fluoride concentration from the concentration of fluoride ion found in each plasma sample. This value "c" represented the concentration of fluoride in plasma arising from the fluoride content of the test or reference water. Any negative values for "c" were treated as zero. The area under the plasma fluoride concentration *vs.* time curve (AUC) from 0 to 3 hrs and 0 to 8 hrs was then calculated.

We "dose-corrected" the AUCs for the F concentration by dividing the AUC value by the concentration of fluoride (mg/L) in the test or reference water, to derive baseline and dose-corrected AUCs for 0-3 and 0-8 hrs, AUC(0-3) and AUC(0-8). In addition, maximum plasma F concentration (c_{max}) and lag time to maximum F concentration (T_{max}) were calculated for each subject for each session.

Statistical Analysis

Following descriptive analysis, the plasma data were analyzed by analysis of covariance with fixed and random effects. The study was treated as a 2-by-2-by-20 factorial design (two types of fluoridation, two levels of hardness, and 20 subjects). Variation between and among subjects was included as a random effect. Type of fluoridation and level of water hardness were fitted as fixed effects. The value of the dependent variable corresponding to the reference water was included as a covariate.

RESULTS

For the total of 100 experimental sessions undertaken overall by the 20 subjects, the mean baseline fasting plasma concentration of fluoride was 19.76 ng.mL^{-1} (SD 3.23 ng.mL^{-1}).

Pharmacokinetic variables were determined for each type of drinking water (Table 1, Fig.): T_{max} ranged from 48.0 min for NFH and NFS waters to 51.7 min for AFS water; c_{max} ranged from 12.5 ng F.mL^{-1} for NFS water to 15.3 ng F.mL^{-1} for AFS water; AUC(0-3) ranged from 973 ng F.min.mL^{-1} (95% CI = 752, 1195) for NFS water to 1217 ng F.min.mL^{-1} (95% CI = 872, 1562) for AFH water, while AUC(0-8) ranged

Table 1. Pharmacokinetics of Fluoride following Ingestion of 500 mL of Test Water or Reference Water

Type of Water		T_{max}[a] (min)	c_{max}[b] ($ng.mL^{-1}$)	AUC(0-3)[c] ($ng\ F.min.mL^{-1}$)	AUC(0-8)[d] ($ng\ F.min.mL^{-1}$)
Naturally fluoridated soft water					
	Mean (95% CI)	48.0 (43.7, 52.3)	12.5 (9.2, 15.7)	973 (752, 1195)	1330 (1005, 1655)
Naturally fluoridated hard water					
	Mean (95% CI)	48.0 (43.1, 52.9)	14.2 (10.7, 17.8)	1058 (793, 1322)	1440 (1071, 1810)
Artificially fluoridated soft water					
	Mean (95% CI)	51.7 (46.9, 56.6)	15.3 (11.7, 19.0)	1167 (918, 1415)	1679 (1284, 2073)
Artificially fluoridated hard water					
	Mean (95% CI)	48.0 (43.7, 52.3)	14.8 (11.6, 18.1)	1217 (872, 1562)	1566 (1175, 1958)
Reference water					
	Mean (95% CI)	48.8 (44.3, 53.2)	14.2 (11.1, 17.3)	1017 (768, 1266)	1328 (991, 1664)

[a] c_{max}: Maximum plasma F concentrations corrected for baseline plasma F and dose (*i.e.*, F concentrations of individual waters).
[b] T_{max}: Time of c_{max}.
[c] AUC(0-3): Dose- and baseline-corrected Area Under the Curve based on positive values of plasma F concentration (c), assuming negative values as zero for the first 3 hrs of the experimental period following ingestion of test waters.
[d] AUC(0-8): Dose- and baseline-corrected Area Under the Curve based on positive values of plasma F concentration (c), assuming negative values as zero for 8 hrs of the experimental period following ingestion of test waters.

from 1328 ng $F.min.mL^{-1}$ (95% CI = 991, 1664) for the REF water to 1679 ng $F.min.mL^{-1}$ (95% CI = 1284, 2073) for AFS water.

Analysis of co-variance (Table 2) showed that, for Area Under the Curve for 0 to 3 hrs [AUC(0-3)], the mean difference between artificially and naturally fluoridated waters was 176.3 ng $F.min.mL^{-1}$ (95% CI = -29.9, 382.5), and that between hard and soft waters was 67.5 ng $F.min.mL^{-1}$ (95% CI = -138.8, 273.7).

For Area Under the Curve for 0 to 8 hrs [AUC(0-8)], the mean difference between artificially and naturally fluoridated waters was 237.3 ng $F.min.mL^{-1}$ (95% CI = -49.7, 524.2), and between hard and soft waters, it was -1.0 ng $F.min.mL^{-1}$ (95% CI = -288.0, 285.9).

No statistically significant differences between waters for T_{max}, c_{max}, or Area Under the Curve for the periods 0 to 8 and 0 to 3 hrs were detected by analysis of covariance.

A noticeable feature observed in the data was the large variation in fluoride absorption both within and between subjects; there were large differences among subjects and, for individual subjects, there were large differences among the different types of water. For three-hour AUC, the estimated standard deviation of the between-subject random errors, based on the analysis of covariance model, was 166 ng $F.min.mL^{-1}$; the estimated standard deviation of the within-subject random errors was 470 ng $F.min.mL^{-1}$. The corresponding figures for eight-hour AUC were 207 and 655 ng $F.min.mL^{-1}$. Across all 20 subjects, the observed differences between the two types of fluoride and between hard and soft water were not large when considered against these two sources of variation and were not statistically significant.

DISCUSSION

This study provides the first data on F pharmacokinetics and bioavailability of F from naturally and artificially fluoridated tap drinking-waters with different degrees of hardness. The results suggest that any differences between waters for these variables are small.

The analytical technique used in this study to measure plasma F concentration has been reported to provide sensitive and reliable determination of F in small volumes of plasma samples (Ekstrand *et al.*, 1977). Since plasma F concentrations are influenced by the relative rates of bone accretion and dissolution, we chose the age range of subjects (21 to 35 yrs) to ensure the inclusion of individuals whose skeletal mass was still increasing. At the same time, the relatively narrow age range selected minimized inter-person variability, thus maximizing the potential of the study to detect treatment-related differences.

The results show similarity in time to reach maximum plasma F concentration for different types of water, which suggests that the rate of F absorption is independent of the type of water with regard to hardness (soft or hard) or fluoride (*i.e.*, artificially or naturally fluoridated).

The relatively wide variations in AUCs for plasma F may have arisen because the subjects were not prevented from moving around during their eight-hour experimental period once the cannula was in place, which may have reduced the rate and extent of gastric emptying, with a resultant impact on

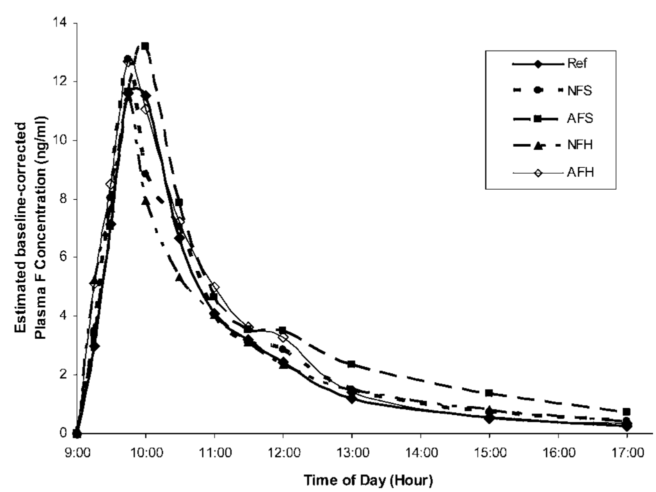

Figure. Estimated baseline-corrected mean plasma F concentration (ng/mL) against time following ingestion of 500 mL of test or reference water (n = 20). Ref, Reference Water; NFS, Naturally Fluoridated Soft Water; AFS, Artificially Fluoridated Soft Water; NFH, Naturally Fluoridated Hard Water; AFH, Artificially Fluoridated Hard Water.

Table 2. Analysis of Covariance using Pharmacokinetic Parameters in Plasma to Compare Artificially Fluoridated Water, Naturally Fluoridated Water, Hard Water, and Soft Water

	Water Comparison	T_{max}[a]	c_{max}[b]	AUC(0-3)[c]	AUC(0-8)[d]
Mean difference	Artificially fluoridated *vs.* Naturally fluoridated	1.9	1.7	176.3	237.3
	Hard *vs.* Soft	-1.9	0.6	67.5	- 1.0
SE of mean difference	Artificially fluoridated *vs.* Naturally fluoridated	1.3	1.1	105.2	146.4
	Hard *vs.* Soft	1.3	1.1	105.2	146.4
95% Confidence Intervals of difference	Artificially fluoridated *vs.* Naturally fluoridated	-0.8, 4.5	-0.5, 4.0	-29.9, 382.5	-49.7, 524.2
	Hard *vs.* Soft	-4.5, 0.8	-1.6, 2.9	-138.8, 273.7	-288.0, 285.9

[a-d] Footnotes as for Table 1.

F absorption from the stomach. Fluoride absorption from the stomach occurs by simple diffusion of HF, with the rate of absorption dependent upon the concentration gradient of HF and inversely related to the pH of the stomach contents (Whitford, 1990). Absorption from the stomach may account for up to 50% of the amount ingested, with the remainder being absorbed from the upper small intestine (Whitford, 1996).

The mean values for c_{max} and AUC for artificially fluoridated waters were slightly, but (given the wide 95% Confidence Intervals) not significantly, higher than those for naturally fluoridated waters. Any differences between F bioavailability of these waters are likely to be small.

With regard to water hardness, although the observed mean AUC for the hard waters was slightly higher than that for the soft waters, any differences between F bioavailability of these waters are also small. This is consistent with results from a study involving administration of F simultaneously with a calcium-rich breakfast and calcium-poor lunch in which the calcium content of the food did not influence F bioavailability (Arnold *et al.*, 1989), although other studies have shown the bioavailability of F to be affected when taken with various foods. Foods containing appreciable amounts of divalent or trivalent cations can lead to the formation of insoluble complexes and precipitates that reduce F absorption (Ekstrand and Ehrnebo, 1979; Spak *et al.*, 1982; Whitford, 1996).

Across all 20 subjects, there were two main sources of variation, with large differences in Area Under the Curve between subjects and, for individual subjects, large differences in Area under the Curve among the 5 different waters. The observed differences between the two types of fluoride and between hard and soft waters were not large when considered against these two sources of variation and were not statistically significant.

In young or middle-aged adults, once F is absorbed from the gastro-intestinal tract, approximately 50% is excreted in the urine (World Health Organization, 1994), although this proportion can vary considerably, depending on numerous variables, including F intake, acid-base balance, and urinary pH (Ekstrand *et al.*, 1978; Whitford, 1996). Some of the relatively wide variation in AUCs between subjects observed in this study might be explained by inter-individual differences in physiological variables such as volume and pH of gastric secretions, gastro-intestinal motility, plasma volume, and urinary pH.

This study adds to the understanding of F pharmacokinetics of naturally fluoridated compared with artificially fluoridated waters, and hard compared with soft waters. The findings suggest that, measured by Area under the Curve, T_{max}, and c_{max} following water ingestion, any differences in bioavailability of fluoride between drinking waters in which the fluoride is present naturally or added artificially, or the waters are hard or soft, are small compared with the large within- and between-subject variation in F absorption following ingestion of drinking waters with F concentrations close to 1 part *per* million.

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