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Bioavailability of fluoride administered as sodium fluoride or monofluorophosphate to humans

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ABSTRACT

A double-bind cross-over study was conducted on four healthy subjects, aged 19–29 years, in order to determine the relative bioavailability and other pharmacokinetics features of fluoride (F) after single oral administration in fasting conditions of 2 mg F as sodium F (NaF) or sodium monofluorophosphate (MFP). The bioavailability was evaluated on the basis of the plasma levels and of the urinary excretion of F. Blood was sampled before and during the 8 h after the administration of the test solutions. For F excretion urine was sampled 12 h before the study and over the 8 h after the administration. Data were tested for statistically significant differences by ANOVA and Tukey's post hoc tests, and also by Student's *t*-test ($p < 0.05$). For the two formulations, the pharmacokinetics of F in plasma was characterized by a rapid absorption and by a peak (C_{max} = 0.1 μg/mL) which was reached 20 min after administration, followed by a biphasic elimination. In the 8 h following the administration the urinary excretion of F accounted for 35–41% of the administered dose, without significant differences between the two formulations. The AUCs (±S.D.) for NaF and MFP were 21.15 (±0.58) and 19.04 (±1.75) min μg mL^{-1}, respectively, and were not significantly different (p = 0.079). Based on the AUC and C_{max} of F in plasma and on the urinary excretion of F during the 8 h following administration, the relative bioavailabilities of the two F formulations were equivalent.

1. Introduction

During the last decades, widespread use of fluoride has led to a decline in caries. However, increased daily ingestion has led to an increase in dental fluorosis [1]. Fluoridated dentifrices have been reported as important risk factors for dental fluorosis [1–8]. This is due to the fact that the amount of ingested dentifrice by children is directly related to the amount loaded onto the tooth brush. The younger the child, the higher the amount of the ingested dentifrice [9]. Most of the dentifrices in the market have sodium fluoride (NaF) or disodium monofluorophosphate (MFP) as active ingredients, but there is no consensus in the literature regarding fluoride bioavailability from them.

Fluoride present in MFP (Na_2PO_3F) is non-ionic, and covalently bound to phosphorus. The absorption of fluoride present in MFP occurs mainly after the enzymatic hydrolysis of the moiety by phosphatases [10]. After an oral dose, fluoride absorption from MFP occurs more slowly than from NaF and other soluble and ionic compounds. This leads to lower and delayed plasma peak fluoride levels. This has been attributed to a relative lack of activity of the acid phosphatase in the gastric mucosa [11,12]. Nevertheless, Rigalli et al. [13] speculated that, in rats, MFP would be absorbed without hydrolysis through the stomach and would bind to plasma globulins forming a MFP-α-2 macroglobulin complex. Inorganic fluoride would be the final product of lysosomal hydrolysis of the protein moiety [14]. Due to this, the authors believe that the bioavailability of fluoride for MFP is higher than that of NaF [15].

Trautner and Einwag [16] described similar bioavailability for MFP or NaF in humans. The authors investigated some variables and observed that peak plasma levels were reduced when the tablets were taken together with food. It was observed that the intake of milk reduced the fluoride availability by 30% compared to the fasting condition, which did not happen when milk was taken

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as part of the breakfast. Due to these results, the authors suggested that "*formation of calcium salts and entrapment of fluoride in coagulation products of milk are important factors causing reduction of fluoride bioavailability. However, prolonged stay of the chyme after concomitant ingestion of food allows fluoride to become liberated from bound forms and coagulation products by digestion processes*".

Additionally, MFP is being used experimentally as an alternative to NaF in the treatment of established osteoporosis with crush fractures for its ability to increase trabecular bone mass. In this regard, MFP offers the advantage of being less irritating to the gastric mucosa [17,12].

Due to the broad use of F in Dentistry and Medicine, and also to the lack of consensus regarding the bioavailability of fluoride as NaF or MFP, the aim of this study was to determine the relative bioavailability and other pharmacokinetics features of fluoride (F) after single oral administration in fasting conditions of the most used formulations (NaF and MFP).

2. Results

Mean plasma fluoride levels at baseline (0.021 ± 0.006 and 0.022 ± 0.013 μg/mL, for NaF and MFP, respectively) did not differ significantly between the formulations.

Table 1 and Fig. 1 show the mean plasma fluoride concentration (μg/mL) as a function of the time of blood sampled after fluoride administration. The differences between the formulations tested at each time point were not statistically significant ($F = 8.185$, $p = 0.64$), but the MFP formulation seems to cause smaller inter-individual variations when compared to the NaF formulation. On the other hand, significant differences were observed among the times of blood sampled ($F = 27.459$, $p < 0.001$). The interaction between both factors (formulations and time) was also statistically significant ($F = 2.151$, $p = 0.032$). Mean peak plasma fluoride concentrations were similar for both formulations and could be seen 20 min after fluoride administration. However, for two of the subjects from the MFP group, peak plasma fluoride levels occurred 40 min after intake (data not shown). Following fluoride administration, plasma fluoride levels were no more significantly different from baseline after 120 and 200 min, for MFP and NaF, respectively. After 480 min, plasma fluoride levels were still slightly higher than the levels found in baseline for NaF.

Mean AUC (±S.D.) was 21.15 (±0.58) and 19.04 (±1.75) min · μg mL^{-1}, for NaF and MFP, respectively, and this difference was not statistically significant ($p = 0.079$). The post hoc calculated statistical power was 75%.

Table 1
Mean plasma fluoride concentration (±S.D., unit μg/mL) as a function of the time of blood collection after administration of 2 mg F as NaF or MFP ($n = 4$)

Time (min)	NaF	MFP
Baseline	0.021 ± 0.006 a	0.022 ± 0.013 a
20	0.106 ± 0.019 j	0.100 ± 0.037 i,j
40	0.080 ± 0.007 h,i	0.095 ± 0.021 h,i,j
60	0.068 ± 0.004 f,g	0.074 ± 0.011 g,h
80	0.072 ± 0.005 g,h	0.062 ± 0.010 f,g
100	0.059 ± 0.001 d,f,g	0.050 ± 0.009 b,c,d,e,f,g
120	0.054 ± 0.003 c,d,e,f,g	0.043 ± 0.001 a,b,c,d,e
160	0.050 ± 0.002 b,c,d,e,f,g	0.031 ± 0.013 a,b,c
200	0.045 ± 0.002 a,b,c,d,e,f	0.036 ± 0.004 a,b,c,e
240	0.040 ± 0.005 a,b,c,d,e	0.032 ± 0.007 a,b,c
300	0.034 ± 0.002 a,b,c	0.036 ± 0.007 a,b,c,e
360	0.035 ± 0.001 a,b,c,e	0.042 ± 0.018 a,b,c,e
420	0.032 ± 0.001 a,b,c	0.027 ± 0.007 a,b,d
480	0.033 ± 0.003 a,b,c	0.020 ± 0.006 a

Values with distinct letters in the same column are significantly different ($p < 0.05$).

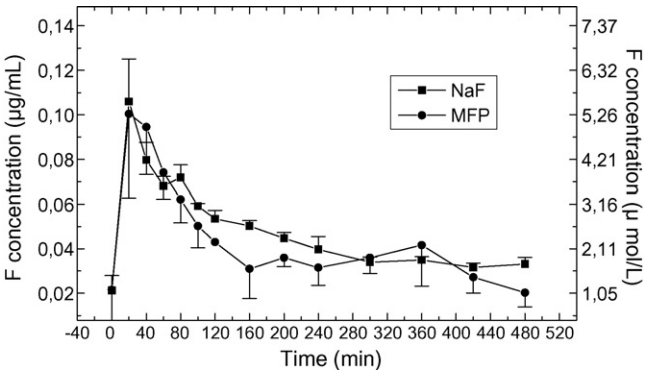

Fig. 1. Plasma fluoride concentrations as a function of the time of blood collection after administration of 2 mg F as NaF or MFP ($n = 4$). Bars indicate S.D.

Mean urinary F output (mg) at baseline and at the experimental day was 0.41 ± 0.26 and 0.71 ± 0.25, respectively, for NaF, and 0.42 ± 0.11 and 0.82 ± 0.29 for MFP. The difference between the two formulations was not statistically significant (data not shown).

The F renal clearance at the experimental day was 33.6 and 43.1 mL/min for NaF and MFP, respectively.

3. Discussion

In this study, a double-bind cross-over study was conducted on healthy subjects in order to determine the relative bioavailability and other pharmacokinetics features of fluoride after single oral administration of NaF or MFP, since there has been controversy in the literature in this regard [10,15,16,20].

The kinetics of absorption of fluoride from the gastrointestinal tract when given as NaF or MFP seemed to be slightly different. For two out of the four subjects, peak plasma fluoride concentrations were seen only after 40 min when MFP was administered, while for all the subjects that received NaF, peak plasma fluoride concentrations occurred after 20 min (data not shown). This resulted in an apparent delay in fluoride absorption when given as MFP, despite peak plasma fluoride concentrations (C_{max}) for both formulations were similar (0.106 ± 0.019 and 0.100 ± 0.037 μg/mL, for NaF and MFP, respectively). It must be pointed out, however, that the calculated post hoc statistical power in this case was very low (only 10%) to allow definitive conclusions on this matter. The fluoride of the MFP molecule is covalently bonded to phosphorus and, thus, it must be released from the molecule by hydrolysis in the small intestine. The relative lack of activity of acid phosphatase in the gastric mucosa results in lower and delayed plasma fluoride levels compared to NaF [11].

As can be seen from Table 1 and Fig. 1, in the present study, the MFP showed slightly higher plasma fluoride levels than the NaF between 40 and 60 min after administration (Fig. 1 and Table 1). After that, slightly lower plasma fluoride levels were found for the MFP when compared to NaF. The urinary fluoride excretion was slightly increased when MFP was used, which may be responsible for the faster plasma fluoride clearance after 60 min when the MFP was used. The delayed MFP absorption and its faster plasma clearance after 60 min of ingestion led to similar plasma fluoride concentrations along the time after administration (AUCs) for both compounds. Similar bioavailabilities of fluoride when administered as NaF and MFP were also reported in humans by Trautner and Einwag [16]. In addition, according to Ekstrand and Ehrnebo [20], Gruninger et al. [21] and Whitford et al. [10], the acute toxicity of fluoride did not differ between NaF and MFP, which gives additional support to the equivalent bioavailabilities of fluoride from both compounds.

In contrast to the results mentioned above, reports in rats [13] and in humans [15] have provided evidence for some absorption of the intact MFP molecule across the gastric mucosa. This fraction that is absorbed intact, would form a complex with α-2-macroglobulin, which would bind to receptors in bone and liver cells, would be uptaken by cells, where it would undergo lysosomal degradation. The fluoride then would return to the extracellular space as ionic fluoride and fluoride bound to low molecular weight macromolecules [14]. This protein-bound fluoride compartment would explain the greater bioavailability of fluoride from MFP than from NaF, which was reported to occur in rats [13] and humans [15]. These authors have reported the bioavailability of fluoride from MFP to be twice as high as that from NaF.

The explanations for the disparate results reported by the present study and the above-mentioned ones [10,16,20,21] in relation to those described by Rigalli et al. [13,15] are not clear. They may be due to the different methods used to analyze fluoride, since in the studies by Rigalli et al. [13,15], HMDS was not used to accelerate the process of diffusion of fluoride, which in consequence required 7 days to occur. In addition, in the study by Rigalli et al. [15], the dose of fluoride given to the volunteers was four times higher than the one used in the present study. Thus, the effect of the dose of fluoride administered on its bioavailability from NaF or MFP should be investigated.

4. Concluding remarks

In conclusion, based on the AUC and C_{max} of fluoride in plasma and in the urinary excretion of fluoride during the 8 h following administration, the relative bioavailability of fluoride from both NaF and MFP is equivalent. This provides additional support for the conception that the professional organizations and regulatory agencies should not endorse the policy of adding greater amounts of fluoride, as MFP, to dental products based on the concept that fluoride in the form of MFP is less hazardous than in the form of NaF.

5. Experimental

5.1. Volunteers

Four healthy female subjects, aged 19–29 years, free of gastrointestinal disorders, renal impairment, and liver disease and without prior F intake took part in the study, approved by the Ethical Committee of Bauru Dental School—University of São Paulo. The purpose of the study was described to the volunteers and written consents were obtained.

5.2. Test substances

In this double-blind study the following formulations were administered, under fasting conditions, in a single dose two-way cross-over design: 2 mL of sodium fluoride (NaF) solution (1.0 mg F/mL; NaF analytical grade; Merck A.G., Darmstadt, Germany) and 2 mL of sodium monofluorophosphate (MFP) solution (1.0 mg F/mL; Purity 97.7%, free F 0.08%, Rhodya, Brazil). The two formulations were equivalent in fluorine content with 2 mg F. The vials containing the solutions drunk by the subjects were washed with 20 mL of deionized water, which was also ingested. There was a wash-out period of at least 3 months between each administration, in order to avoid any problems related to blood sampled.

5.3. Experimental procedure

The subjects fasted for 12 h (overnight) before the day of the experiment. All experiments began at 8.00 a.m. During the study period of 8 h no foods and drinks were allowed to the subjects, except for a standard snack (one loaf of bread with butter and a cup of sugarless coffee, prepared with deionized water) that was offered at 11.00 a.m. The fluoride content of the snack was 0.018 and 0.022 mg fluoride for the first and second experiments, respectively.

5.4. Blood collection

For each experimental day, a total of 14 blood samples were taken from the arm vein into tubes containing 5 μL of heparin (containing 0.184 μg F/mL). Sampling was done before (baseline) and during 8 h after administration. After administration, blood was sampled at 20 min intervals for 2 h, at 40 min intervals up to 4 h and then at 1-h intervals up to 8 h. The samples were centrifuged at 3000 rpm (Jouan A14) for 5 min, and plasma was stored at −20 °C until F analysis.

5.5. Urine collection

Urine was sampled 12 h before (baseline) and over the 8 h after the administration. The subjects were instructed to void their urine only in their individual labeled wide-necked plastic flasks. Urine sampled (baseline) started at 8:00 a.m. and flask 1, containing all the urine sampled up to 8:00 p.m., was closed and brought to our laboratory, where volume and pH of each individual sample were immediately determined, while an aliquot (50 mL) was frozen (−20 °C) until fluoride analysis, which was carried out within the next 48 h. Flask 2 contained all the urine voiding from 8:00 a.m. to 4:00 p.m. (experimental day). All of the flasks containing urine samples were kept permanently closed in a refrigerator until they were brought to the laboratory.

5.6. Analytical procedure

5.6.1. Plasma

The samples were prepared with previously heated F-free H_2SO_4 in order to remove CO_2, before the diffusion was conducted. The plasma F concentrations were determined in duplicate after overnight, hexamethyldisiloxane (HMDS)-facilitated diffusion [18] as modified by Whitford [19] using a fluoride ion-specific electrode (Orion Research, Cambridge, MA, USA, model 9409) and a miniature calomel reference electrode (Accumet, #13-620-79), both coupled to a potentiometer (Orion Research, model EA 940). Fluoride standards (0.25, 0.50, 1.00, 5.00 and 10.00 nmol) were prepared in triplicate and pre-diffused with F-free H_2SO_4 in the same manner as the samples by serial dilution of a stock standard containing 0.1 M fluoride (Orion 940906). Comparison of the millivolt readings demonstrated that the F in the diffused and pre-diffused standards had been completely trapped and analyzed (recovery > 99%). The millivolt potentials were converted to μg F using a standard curve with a correlation coefficient of $r \geq 0.99$.

5.6.2. Urine

Fluoride analyses in the urine samples were made by the direct method, using the ion-specific electrode (Orion Research, Cambridge, MA, USA, model 9409), after sample buffering with an equal volume of TISAB II. A set of standards (containing 0.05, 0.10, 0.20, 0.40, 0.80 and 1.60 ppm fluoride) was prepared, using serial dilution from a 100-ppm NaF stock solution (Orion). The millivolt potentials were converted to ppm F using a standard curve with a correlation coefficient of $r \geq 0.999$. The mean repeatability of the readings, based on the duplicate samples, was 96%. The same person (MARB), who was unaware of the volunteers from which samples were taken, made all the analysis.

5.7. Calculation

Fluoride absorption was measured by change in the area under the curve (AUC). The AUC was calculated after integration of the graph, using the software Microcal Origin version 6.0 (Microcal Software, Inc., Northampton, MA, USA). The plasma F peak level (C_{max}), the time needed to reach this level, and the time until the elevated plasma fluoride levels decreased to basal levels were evaluated graphically.

5.8. Statistical analysis

Differences in plasma fluoride concentrations and in the amount of fluoride excreted from urine were tested by two-way repeated measures analysis of variance, in which one factor was the compound tested and the other was the sampling time. Tukey's test was selected as the post hoc test for the analysis of variance. A significance level of 0.05 was selected a priori. Differences in the area under the plasma F concentration versus time curve were assessed by paired *t*-test ($p < 0.05$).

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