Influence of vitamins and iron on plasma fluoride levels in rats

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Abstract

Thirty female Sprague-Dawley rats (250 ± 10 g) were divided randomly into five equal groups. After overnight fasting, a silastic catheter was placed in the jugular vein of each rat. Each group was intragastrically administered 0.25 mg F/250 g rat weight in 1 ml of one of the following forms of fluoride supplements: Pediaflor® (Abbott/Ross, Columbus, OH), Tri-Vi-Flor®, Tri-Vi-Flor + iron; Poly-Vi-Flor® or Poly- Vi-Flor + iron (Mead Johnson Nutritionals, Bristol-Myers Squibb Co., Evansville, IN). Timed blood samples were collected and plasma fluoride concentration was determined using the microdiffusion method. The presence of iron and vitamins affect the bioavailability of fluoride as measured by the area under the time-plasma fluoride concentration curve. (Pediatr Dent 14:37–40, 1992)

Introduction

Ever since the discovery of the anticariogenic property of fluoride, various forms and vehicles for topical or systemic delivery of fluoride have been marketed.[1] Recommendations from the Council on Dental Therapeutics of the American Dental Association (ADA) suggest that children younger than 13 who reside in communities with water fluoride concentrations less than 0.7 ppm should be supplemented with dietary fluoride.[2] Fluoride supplement may be dispensed in liquid or tablet form and may come in combination with various vitamins with or without iron.[3]

Gastric absorption of fluoride is known to be affected by dietary factors[4, 5], especially by the presence of divalent or trivalent cations.[6] Furthermore, absorption of fluoride in the stomach is favored by an acidic environment wherein fluoride exists as hydrogen fluoride, a molecular form which facilitates transport through the gastric mucosal membrane.[7] Ascorbic acid, thiamine hydrochloride, nicotinic acid, and pyridoxine hydrochloride, among many others, are vitamins which produce an acidic solution when dissolved in an aqueous medium.[8]

This study was designed to evaluate the effect of iron and different vitamins in the various forms of liquid fluoride supplement on the bioavailability of fluoride in an animal model.

Materials and Methods

Thirty female Sprague-Dawley rats (250 ± 10 g) were housed in clear plastic cages in a room that was maintained at 23 ± 1°C and were fed tap water and Rodent Laboratory Chow 5001® (Purina Mills, Inc., Richmond, IN) ad libitum. This rodent food contains 76% total digestive nutrients, 23.4% protein, 4.5% fat, 35 ppm fluoride, 1.00% calcium, 0.61% phosphorus, 15 ppm thiamine; 8.0 ppm riboflavin, 95 ppm niacin; biotin 0.07% ppm; vitamin A 15 IU/gm, vitamin D 4.5 IU/gm, and vitamin E 40.0 IU/gm. A 12-hr light cycle (6 AM to 6 PM) was maintained in the animal rooms. Under general anesthesia a cocktail was placed in each rat via a silastic jugular vein catheter (.02 ID x .045 OD).[9] The cocktail contained 15.6 mg of ketamine, 0.6 mg of Acepromazine® (Ayerst Laboratory, Inc., New York, NY) and 3.1 mg of Rompun® (Lloyd Laboratory, Shenandoah, IA) in each ml of solution. These catheters exited dorsally behind the head and were filled with heparinized normal saline solution (1000 IU heparin/30 ml of saline solution) to prevent clotting and blockage of the catheters. The animals were allowed 24 hr to recover from the surgical procedure before the fluoride supplements were intragastrically administered. During the recovery period, only water was available to the rats. Groups of six rats were prepared surgically as described above to ensure that five rats always were available for experimentation. These rats were weighed and given a dose of 0.25 mg ionic fluoride/ml solution/250 g body weight (ionic fluoride concentration of each preparation is verified by direct F reading with a F specific electrode) or one of the following fluoride supplements intragastrically: Pediaflor® (Abbott/Ross, Columbus, OH); Tri-Vi-Flor®; Tri-Vi-Flor + iron; Poly-Vi-Flor®; Poly-Vi-Flor + iron (Mead Johnson Nutritionals, Bristol-Myers Squibb Co., Evansville, IN). Blood samples (0.2 ml) were collected through the jugular vein catheter, in heparinized, 1-ml plastic disposable syringes at 0, 20, 40, 60, 90, 120, and 180 min after the intragastric doses. The blood samples were transferred to polyethylene microcentrifuge tubes, and plasma samples were removed and stored at 4°C for fluoride determination that was performed with an inverted Orion fluoride-specific electrode and millivolt meter (Model 811) following HMDS diffusion.[10] Area under the curve of time versus plasma fluoride concentration was calculated using the trapezoidal rule. Student's *t*

test was used to determine whether the difference between the groups were statistically significant.

Results

The formulations of the four vitamin-containing fluoride supplements are listed in the Table. All the fluoride supplements contained 0.25 mg fluoride per ml. Tri-Vi-Flor contains fluoride and vitamins A, D, and C, while the Poly-Vi-Flor contains additionally, vitamins E, thiamine, riboflavin, niacin, and vitamin B6. Vitamin B_{12} is not present in Poly-Vi-Flor with iron because of the undesirable interaction between vitamin B_{12} and iron.

Results in Fig 1 (next page) showed that when rats were given Pediaflor, the plasma fluoride level rose rapidly and peaked about 20 min following intragastric feeding. Plasma fluoride decreased over the next hour; however, it remained significantly above the baseline value before fluoride was given, even 3 hr after fluoride intake. When the same dose of fluoride (0.25 mg F per 250 g rat weight) was given as Tri-Vi-Flor, plasma fluoride also peaked at 20 min after fluoride intake. The peak plasma fluoride level was higher than 34.0 μM; this is twice the value of the peak-plasma fluoride level reached by rats given fluoride alone as Pediaflor (14.6 μM). Plasma fluoride levels of rats receiving fluoride as Tri-Vi-Flor remained significantly higher than those which received fluoride as Pediaflor throughout the 3-hr experimental period.

When the same dose of fluoride was given as Tri-Vi-Flor with iron, plasma fluoride levels of the rats exhibited a similar pattern as those which received Pediaflor over the 3-hr experimental time, except that at each sampling point, the plasma fluoride level was lower. However, such differences were not statistically significant.

Plasma fluoride levels of rats given 0.25 mg fluoride per 250 g body weight as Poly-Vi-Flor peaked (20.9 μM) at 20 min after fluoride intake, a level 43% greater than that reached by animals given Pediaflor alone (14.6 μM, Fig 2, next page). Plasma fluoride levels of these rats remained significantly greater than rats given Pediaflor throughout the 3-hr experimental period. When Poly-Vi-Flor plus iron was given to the rats, the peak plasma fluoride level (18.5 μM) was reached at 20 min after fluoride intake and was slightly lower than that of Poly-Vi-Flor (20.9 μM) but greater than that which resulted from intake of Pediaflor (14.6 μM).

Comparison of the area under the time-plasma fluoride concentration curves showed that Tri-Vi-Flor plus iron and Poly-Vi-Flor plus iron drops did not differ statistically from Pediaflor. Tri-Vi-Flor drops-and Poly-Vi-Flor drops showed values of 78 and 62% respectively, significantly ($P < 0.02$) greater than that of fluoride drops (Fig 3, next page).

Discussion

Results in Fig 1 demonstrate that plasma fluoride levels in rats that were given fluoride as Tri-Vi-Flor drops (contains fluoride and vitamins A, D, and C) were significantly higher than those in rats that were given fluoride as Pediaflor drops. Further experiments will be needed to identify the vitamins which are responsible for this greater plasma fluoride level. In guinea pigs, it has been reported that added increments of ascorbic acid increased retention of fluoride in the skeleton and soft tissues.[11] It has been suggested that vitamins A, C, and D tend to mitigate the symptoms of fluorosis and retard the development of fluoride toxicosis, with ascorbic acid possessing the greatest effect.[12] Thus, retention by the skeleton may be one of the protective mechanisms employed by the animal against the toxicity of fluoride.[11] All of the previously reported studies were chronic investigations and did not record blood plasma fluoride levels, which makes any comparison to the present investigation impossible. Although the elevated fluoride levels in rat plasma

Table. Contents of fluoride supplements with vitamins*

	Tri-Vi-Flor	*Tri-Vi-Flor with iron*	*Poly-Vi-Flor*	*Poly-Vi-Flor with iron*
Vitamin A, IU	1500	1500	1500	1500
Vitamin D, IU	400	400	400	400
Vitamin E, IU	—	—	5	5
Vitamin C, mg	35	35	35	35
Thiamine, mg	—	—	.5	.5
Riboflavin, mg	—	—	.6	.6
Niacin, mg	—	—	8	8
Vitamin B6, mg	—	—	.4	.4
Vitamin B12, mg	—	—	2	—
Iron, mg	—	10	—	10
Fluoride, mg	.25	.25	.25	.25
pH value	5.7 – 6.1	2.8 – 3.2	2.9 – 3.6	2.9 – 3.2

* All contents are based on those in 1 ml of the fluoride supplement. Fluoride supplements reported here are products of Mead Johnson & Co., Evansville, Indiana 47721, USA.

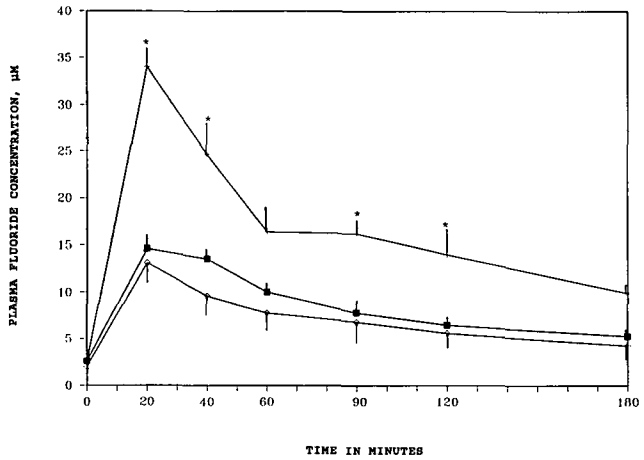

Fig 1. Plasma fluoride concentration in female SD rats following ig administration of 0.25 mg F/250 g body weight in form of Pediaflor -■-, Tri-Vi-Flor -+-, or Tri-Vi-flor with iron -◇-. Each point on the curve represents the average value of five rats, with the SEM indicated by vertical bars.
*Values significantly different from the Pediaflor ($P < 0.05$).

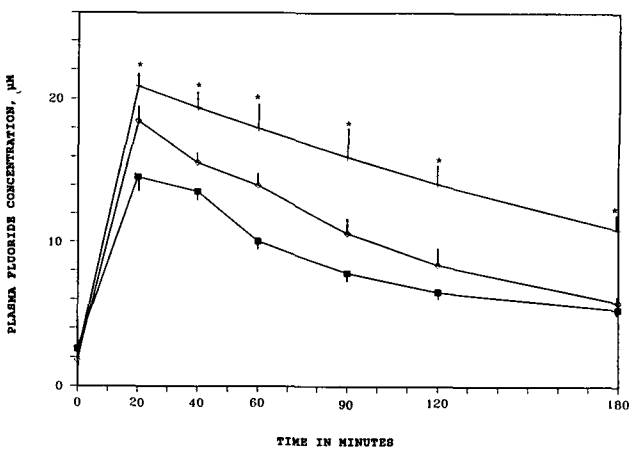

Fig 2. Plasma fluoride concentration in female SD rats following ig administration of 0.25 mg F/250 g body weight in form of Pediaflor -■-, Poly-Vi-Flor -+-, or Poly-Vi-Flor with iron -◇-. Each point on the curve represents the average value of five rats, with the SEM indicated by vertical bars.
*Values significantly different from the Pediaflor ($P < 0.05$).

could explain the greater fluoride retention in bones of rats and guinea pigs reported in earlier studies, reduction of renal fluoride clearance also could result in an elevated plasma fluoride level.

Results shown in Fig 1 also demonstrate that ferrous iron is responsible for the lower plasma fluoride levels in rats that were given Tri-Vi-Flor plus iron drops for at least 3 hr following intragastric administration of fluoride-containing vitamin suplements. Based on skeletal uptake of fluoride during a six-week chronic investigation, it was reported that iron did not affect fluoride bioavailability in male rats.[13] The same study also reported that a significant effect of fluoride absorption and retention was observed in these male rats during week six of the study. Using weanling female Wistar rats, however, it was discovered that fluoride retention in the femur was 24% less in rats fed vitamin-mineral supplements containing 46.94 mg ferrous fumarate per 10 g diet when compared to control rats.[14] The presence of ferrous iron seems to decrease fluoride bioavailability.

Results in Fig 2 show that Poly-Vi-Flor exhibited a similar augmentative effect on plasma fluoride levels in female Sprague-Dawley rats. In addition to vitamins A, D, and C, B vitamins also are present in Poly-Vi-Flor. Although information on the acute effect of B vitamins on fluoride metabolism is not available, chronic feeding studies on male Wistar rats have shown that at near optimal doses, neither riboflavin[15] nor thiamine[16] has much of an effect on skeletal fluoride retention. However, at higher doses of twice the minimal daily requirement level, intake of thiamine resulted in 15% less net skeletal fluoride accretion.

Fluoride absorption in the stomach is known to be favored by an acid environment.[7] However, results in this study indicate the presence of a divalent cation, iron, will be more than sufficient to antagonize the influence of acidity of the preparations. Plasma fluoride level is lower in rats fed Tri-Vi-Flor with iron drops

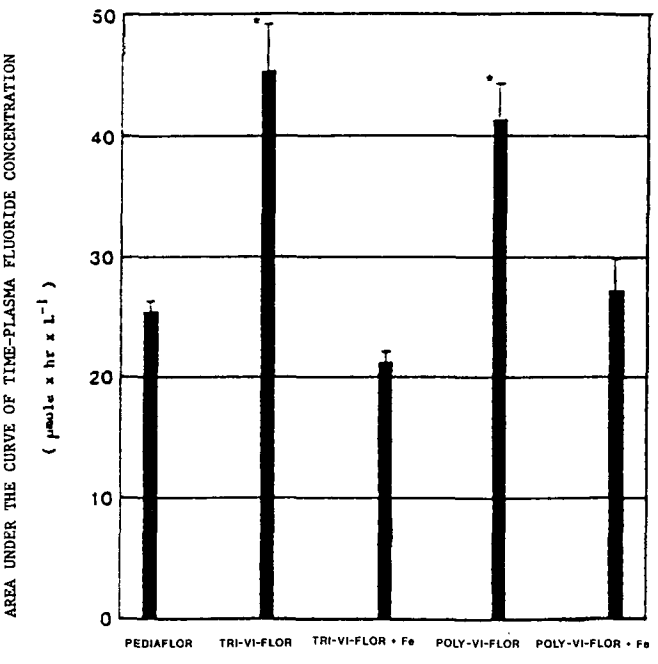

Fig 3. Area under the time-plasma fluoride concentration curve of female SD rats following ig administration of 0.25 mg F/250 g body weight as Pediaflor, Tr-Vi-Flor, TriVi-Flor with iron, Poly-Vi-Flor, or Poly-Vi-Flor with iron. Each column represents the average value of five rats, with the SEM indicated by vertical bars.
*Values significantly different from the Pediaflor ($P < 0.05$).

than rats fed Tri-Vi-Flor even though the former fluoride preparation has a much lower pH (Table).

The present studies have shown that plasma fluoride level is significantly influenced to a large extent by the presence of vitamins and iron. However, when interpreting the present data, one should bear in mind that retention of fluoride depends upon the age, fluoride content of the skeleton and the intake. The rats used in this study were about 10 weeks old and were still growing actively. Further studies will be necessary to establish the relationship, if any, between the acute effect of vitamins on plasma fluoride level and the retention of fluoride in mineralized tissues.

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High-fat diets and cavities

High fat diets can not only lead to cholesterol problems, but also may increase the chances of developing dental caries, according to a collaborative study between two dental schools.

Dental researchers in New Jersey and Washington State have found that patients on high-fat diets have higher levels of lipids — fatty substances — in their saliva than those on low-fat diets. These same people had more cavities.

Researchers are attempting to determine why the high content of fats in saliva coincides with the high rate of cavities. They also are trying to develop a simple saliva test to measure cholesterol levels reliably as an alternative to blood sampling.