BULLETIN OF TAIPEI MEDICAL COLLEGE, VOL. 15, MAY 1986
P. 103-110

Influence on Growing Rats with Perinatal Fluoride Administration. Part 1.

BEY-RONG GUO, SCHING-JOUNG LIN and CHE-TONG LIN

ABSTRACT

The effects of differing perinatal concentrations of NaF in water on juvenile rat molar morphology and tissue response was studied. Thirty-two Long-Evens pregnant rats were equally divided into 4 groups: Control group I dams drank deionized water; group II drank a concentration of 1 ppm NaF; group III took 6 ppm and group IV, 12 ppm NaF. All dams were fed the same diet. Their 44 offspring were scarified at 19 days and the right first mandibular molar of each was extracted, measured and sectioned. Statistical analyses of the measurements showed significant differences among the overall sizes and shapes of the molars. The teeth of the offspring of group II, III and IV accumulated significantly ($P < 0.05$) greater amounts of fluoride than group I's offspring. Sectioned left molar teeth were observed under a ploarized microscope to have more compacted enamel tissue in group III and IV. A series of macroscopic and microscopic measurements of femoral bones showed no significant difference among four dams. Furthermore, no evidence of pathologic change on liver and kidney of all offsprings when available for microscopic examination.

INTRODUCTION

There have been many reports about the effect of fluoride on tooth morphology. Investigators in New Zealand(1) found that the diameters of fluoridated teeth were about 2% smaller and the depth of fissure was about 5% smaller than in non-fluoridated teeth.

In rats with 6 ppm fluoride in their diet(2), the size of molars was reduced and the proportion of molars with rounded fissures was higher than in the controls. Further, reduction in the thickness of enamel and dentine was observed(3,4). A concentration of 8-9 ppm fluoride was necessary to cause a loss of pigment from the rat incisor(5).

Further, the use of an electron microscope to determine the effect of different levels of fluoride on the ultra-structure of ameloblasts in the rat has been re-

School of Dentistry, Taipei Medical College.
Received for Publication: December 30, 1985.

ported[4]. The ameloblasts showed great changes in synthesis at high concentrations of fluoride.

This experiment was designed to investigate statistically the effects on their molars of fluoride administered to pregnant rats and newly born rats in an experimental group, using non-fluoridated group as controls.

The following points were considered:

a. The significance of fluoride on mesio-distal diameter changes on rat molars.
b. The modification in pit and fissure depth.
c. Whether fluoride content in teeth increases in correspondence with perinatal fluoride administration.
d. Prolonged high fluoride intake whether will affect bones and visceral organ.

MATERIALS AND METHODS

Administration of Fluoride

Thirty-two Long-Evens pregnant rats of 230±10g body weight were selected. They were randomly assigned to groups I, II, III or IV and given deionized water, or deionized water with fluoride concentrations of 0.004mg/kg, 0.004x6mg/kg or 0.004x12mg/kg respectively. A specially-designed 18-gauge needle with a bulbous tip was attached to a 1ml syringe for the daily feeding[6]. The animals were weighed daily and the dosage was accordingly adjusted for gains in weight. This procedure was begun from the third day of the rat's pregnancy to the sacrifice of juvenile rats which were 19 days after birth. To ensure that the concentration of fluoride in the plasma was at a constant level, drinking water provided was also deionized; the fluoride ingestion each day was thus strictly controlled. Nutritional requirements were also standardized by feeding the rats of all groups the same diet (Rodent Laboratory Chow 5001). The 44 offsprings of the pregnant rat provided for the study after the rats were sacrificed by decapitation under ether anesthesia.

(A) Measurement of Mesio-distal Dimensions and Fissures:

Measurements of samples were undertook under double blind procedure. The teeth to be measured were placed on a stereomicroscope to record the distance between distal contour point and mesial contour point. A very fine needle was used for fissures, and inserted vertically to the central fossa corresponding the highest cusp of the first right mandibular tooth. The needle was bent at that point, and its length recorded by micrometer to establish fossa depth.

(B) Observation of Ground Sections of Rat Molar Teeth:

Each first left mandibular molar was ground sectioned in 100μ; the tooth section was observed with light microscope, and then immersed in water for refraction to facilitate observing, the enamel arrangement under a polarized microscope.

(C) Fluoride Content Analysis

The first right mandibular molar of each animal to be studied was placed in a Pyrex beaker where dry ashing took place over 48 hours in the muffle furnace at 500°C. After the beakers were cooled nitric acid was added to dissolve the tooth ash. The following steps were then taken:

1. Preparation of standard curve:
   Fluoride standards were prepared in the range 0.00 to 2.50mg by diluting appropriate amounts of standard fluoride solution with distilled water to 100ml. Using a pipet, 5.00ml of alizarin red solution and 5.00ml of zirconium-acid solution were added to each standard, mixed well; the reaction was then allowed to proceed for 60±2 min. The photometer was set to zero absorbance (100 per cent transmittance) with distilled water. At the end of 60±2 min, a reading was taken on each standard, using a wavelength in the 520 to 550 nm range. A curve of fluoride-absorbance relationships was then plotted. A new standard curve was required whenever a fresh batch of either of the two reagents was prepared or when a different room temperature occurred.
2. Sample pretreatment:
   If a sample contained residual chlorine, it could be removed by adding 1 drop (0.05ml) of arsenite solution for each 0.1mgCl, and mixing.
3. Color development:
   A 100 ml sample or an aliquot diluted to 100 ml was used. The temperature of the sample was adjusted to the value used for the standard curve. After 5.00 ml zirconium-acid solution was added, the whole was mixed well. After 60±2 min and then setting the reference point of the photometer as was done for the standard curve, the absorbance was read.

(D) Measurements of Length and Weight in Terms of Femoral Bones

The femoral bones were removed from rat offsprings and soft tissue attached were stripped off. The series of length and weight measured for each bone using accurate gauge and eletro-balance. The entire sample were recorded and tabulated according to each dam.

(E) Histologic Observations for Femoral Bone, Liver and Kidney

All the speciments were fixed in 10% formalin, decalcification with 3% trichloroacetic acid for femoral bone, embedded in paraffin and cut in 6 μ section. After staining with hematoxylin and eosin, the sections were examined under the light microscope thoroughly.

RESULTS

(A) Data in Table 1, the perinatal supply of fluoride did alter significantly the size of first right mandibular molars of rats. There seemed a sharp reduction between II and III. The result had statistically significance in III and IV which received 6ppm and 12ppm respectively. Group II did not change. ($P > 0.05$).

In Table 2, a positve correlation shows the effect of fluoride on depths of fissures. The control group had the most deep fissures, followed by II, III and IV. But only III and IV were significant as compared with the control group ($P < 0.05$).

(B) The thickness of enamel at the base

(A) **Table 1. The Effect of Fluoride on the Size of Right Mandibular Molar Teeth of Rat Offspring**

	Mean mesio-distal dimension/mm*	S.D.	t	P
Group I	47.9	1.76	/	/
Group II	47.0	1.10	1.064 (no sig.)	$P > 0.05$
Group III	45.68	1.30	3.365 (sig.)	$P < 0.05$
Group IV	45.5	0.50	4.350 (sig.)	$P < 0.001$

*The mean mesio-distal dimension is 30 times magnified under stereomicroscope. Statistical method is Student's t-test.

(B) **Table 2. The Effect of Fluoride on the Pit and Fissure of Right Mandibular Molar Teeth of Rat Offspring**

	Depth of pit & fissure (mm)	S.D.	t	P
Group I	1.147	0.0129	/	/
Group II	1.126	0.0143	1.998 (no sig.)	$P > 0.05$
Group III	1.116	0.01	6.299 (sig.)	$P < 0.001$
Group IV	1.088	0.12	10.800 (sig.)	$P < 0.001$

*Statistical method is Student's t-test.

(C) **Table 3. Concentrations of Fluoride (ppm) in Ash of the Molar Molars of Rat Offspring**

Group	Mean of fluoride in rat molar teeth	S.D.	n	t	P
I	31.256	4.100	11	/	/
II	34.126	6.543	11	2.171	< 0.05
III	37.032	2.994	11	2.629	< 0.05
IV	42.620	3.204	11	7.244	< 0.05

*Statistical method is Student's t-test.

of fissure were not alteration significantly when ground sections were observed thoroughly, but the ground sections immerse in water showed stronger negative bifrigence in group III and IV.

(C) Table 3 records the results of analyses of teeth of rat offspring in each group. The experimental groups are compared to control group I. A gradual increase of fluoride was seen. Note that even group

II was significant.

(D) Tested by Kruskal-Wallis one-way analysis of variance by ranks, it was found that both of length and weight of femoral bones were no significant differences in four groups of rats.

(E) All speciments of femoral bones revealed nearly equivalent osteablasts activity, thickness of cartilage plate and number of osteoclasts. Mild congestion of kidney and liver were the predominant finding of sections thoroughly.

DISCUSSION

The size of the crown of the rat molars reduced significantly under the conditions of this experiment. While Gray stated there was no statistically significant reduction in the molars of rat offspring[7], Paynter and Grainger[2] found that the sucklings of dams drinking water if 12 ppm concentration had molars which shorter mesio-distally. Kruger[4] confirmed this, observing a reduction in the thickness of enamel and dentine, with smaller teeth resulting. The result of difference is possible that the fluoridated water proffered to each rat was not completely consumer, so the fluoride concentration might not have reached the critical value. The next reason possibly accounting for the different results might be the difference in strains of rat used. Gray used New Zealand Wistar Whites, Paynter and Grainger used Carworth; Long-Evens were used in this experiment.

The depth of pit and fissure is an important factor in frequency of caries. Painter and Grainger's results[2] agreed with this experiment in pit and fissure depths, although they reported the proportion of molars with rounded fissures to be higher in fluoridized rats than in the controls. Kruger and Gray[3,4] observed only wider fissures. Since Kurger injected fluoride intraperitoneally into suckling rats, a concentration of fluoride approaching the critical intracellular level may have resulted.

No matter if the fissures became wider or turned more shallow, there is benefit in caries prevention. The food debris is easier to remove. The delayed eurption and mottled enamel was not be observed in four dams. Glenn[8-13] proved clinically that fluoride with prenatal supplementation caused shallow pits and fissures and a reduction of caries on deciduous teeth. The fluoride uptake is important during the maturation phase of enamel. Rats teeth occur later than phase of human teeth, so that post-natal supplement is rational in animal model. Furthermore, developing human teeth are more sensitive to fluoride than are rat teeth, the low level of fluoride is enough to effect.

From the fluoride content analysis of group II, fed only 1 ppm of NaF per day, showed statistical significance. This implies that fluoride was actively incorporated into developing teeth. The configuration of enamel is stronger and more resistant to caries. For group III and group IV, with an even higher fluoride level, this is an expectation.

According to the data of femoral bone, author believed it can't effect the growth of bone even high fluoride supplement in the limited time. The manifestation of fluoride intoxication including osteosclerosis and pathological change of liver or kidney was not presented in all series of histological sections. It seems that there is no tendency for any side-effect in the high fluoride than controls.

Under the polarized microscope, all four groups were compared. When maternal rats were fed a higher concentration of fluoride, the teeth of the offspring showed very obvious negative birefrigence, an indication of more compacted enamel tissue.

Papers about the use of prenatal or perinatal fluoride supplementation were published, but only a few studies evaluated the caries prevetion effects of perinatal or prenatal exposure to watere-borned fluoride. Three studies supported the position that perinatal or prenatal exposure to fluoridated drinking water benefits primary teeth[14,15,16]. The rest of the studies denied the effect of fluoride in caries prevention[17,18,19,20,21]. The present experiment is a cohort study which can support the previous view. But, the actual mechanism involved in the fluoride effects requires further investigation.

Concluded the above points, the perinatal administration of fluoride will alter teeth morphology and component. All these had benefit in caries prevention.

ACKNOWLEDGEMENTS

I wish to thank Professor Tong-Jung Hsu, Associate Professor E.E. Chang, and Instructor Fong-Wei Wang, all of Taipei Medical Colledge, Department of Analytic Chemistry for the fluoride analyses of teeth, as well as to Professor Ming-Jer Shieh, School of Nutrition and Health Science, and Mr. Yang-Yeh Shau who kindly housed the laboratory animals used in this study.

REFERENCES

1. COOPER VK, LUDWING TG: Effect of fluoride and of soil trace elements on the morphology of permanent molars in man. NZ Dent. J. 61; 33-40, 1965.
2. PAYNTER KJ, GRAINGER RM: The relation of nutrition to the morphology and size of rat molar teeth. J. Can. Dent. Assoc. 22; 519-531, 1956.
3. KRUGER BJ: Ultrastructural changes in ameloblasts from fluoride treated rats. Arch. Oral. Biol. 13; 969-977, 1960.
4. KRUGER BJ: The effect of different levels of fluoride on the ultrastructure of ameloblasts in the rat. Arch. Oral. Biol. 15; 109-114, 1970.
5. PINDBORG JJ: Disturbances in tooth formation in "Pathology of the Dental Hard Tissues". 1st ed. W.B. Saunders. Philadelphia, pp. 108-114, 1970.
6. NAVIA JM: Animal Models in Dental Research. Birmingham, The University of Alabama Press, pp. 149, 1977.
7. GRAY HS: A morphological study of the influence of fluoride on rat molar teeth. Archs. Oral. Biol. 18; 1451-1460, 1973.

8. GLENN F: Immunity conveyed by a sodium fluoride supplement during pregnancy. J. Dent. Child. 44; 391-395, 1977.
9. GLENN F: Immunity conveyed by a sodium fluoride supplement during pregnancy. Part II. J. Dent. Child. 46; 17-24, 1979.
10. GLENN F: The rationale for the administration of a NaF tablet supplement during pregnancy and postnatally in a private practice setting. Part III. J. Dent. Child. 48; 18-22, 1981.
11. GLENN F, GLENN W, DUNCAN R: Fluoride Tablet supplementation during pregnancy for caries immunity: A study of the offspring produced, Part IV. Am. J. Obstet. Gyne. 143; 560-564, 1982.
12. GLENN F, GLENN W, DUNCAN R: Prenatal fluoride tablet supplementation and improved molar occlusal morphology. Part V. J. Dent. Child. 51; 19-23, 1984.
13. GLENN F, GLENN W, DUNCAN R: Prenatal fluoride tablet supplementation and the fluoride content of teeth. Part VIII. J. Dent. Child. 51; 344-351, 1984.
14. ARNOLD F et al: Effect of fluoridated public water supplies on dental caries prevalence. Pub. Health Report. 71; 652-658, 1956.
15. BLAYNEY JR, HILL IN: Evanston dental caries study. XXIV. Prenatal fluorides-value of waterborne fluorides during pregnancy. J. Am. Dent. Assoc. 69; 291-294, 1964.
16. TANK C, STORVICK CA: Caries experience of children one to six years old in two Oregon communities (Corvallis and Albnay). I. Effect of fluroide on caries experience and eurption of teeth. J. Am. Dent. Assoc. 69; 749-757, 1964.
17. CARLOS JP, GITTELSON AM, HADDON W: Caries in deciduous teeth in relation to maternal ingestion of fluoride. Pub. Health Report. 67; 658-660, 1952.
18. HOROWITZ HS, HEIFETZ SB: Effects of prenatal exposure to fluoridation on dental caries. Pub. Health Report. 82; 297-304, 1967.
19. KATZ S, MUHLER JC: Prenatal and postnatal fluoride and dental caries experience in deciduous teeth. J. Am. Dent. Assoc. 76; 305-311, 1968.
20. LEMKE CW, DOHERTY JM, ARRA MC: Controlled fluoridation: the dental effects of discontinuation in Antigo, Wisconsin. J. Am. Dent. Assoc. 80; 782-786, 1970.
21. LEWIS DW, et al: Initial dental care time, cost and treatment requirements under changing exposure to fluoride during tooth development. J. Can. Dent. Assoc. 4; 140-144, 1972.

氟化物對懷孕及幼年期大鼠之影響
第一報

郭倍棠　林興永　林哲堂

本研究旨在觀察將懷孕時與出生後幼年期之大鼠，施以不同濃度的氟化鈉後，對幼年鼠之臼齒外型與構造產生之影響以及身體組織對不同濃度氟化鈉下的反應，首先將32隻懷孕的Long-Evans種的大鼠分成4組：第1組爲對照組，使用去離子水來餵食；第2組使用含有1ppm濃度的氟化鈉；第3組含6ppm，第4組含12ppm。各組的大鼠都餵以相同的飼料。44隻各組所生之幼鼠，在出生後19天將之犧牲，並將其下顎右側第1臼齒拔除之，經測量其大小和外型後，顯示有意義的差異，同時2、3、4組牙齒氟化物的含量亦高於第1組（$P<0.05$）。將各組左側之臼齒作成磨片，浸水後使用偏光顯微鏡觀察時，在第3、4組的磨片中，可見較緻密的牙釉質。而後，將4組幼年鼠的脛骨取出，測量其大小、重量，以及施以組織切片觀察，皆無有意義的差異。而且，在所有幼鼠之肝、腎組織切片中，亦不見有中毒之病變發生。

台北醫學院牙醫學系
民國七十四年十二月卅日受理