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Effect of Prenatal Fluoride on Bone Compositions of Rat

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1. Introduction

Over the past 50 years extensive researches throughout the world consistently demonstrated the safety and efficacy of fluoride in preventing dental decay[1-4]. During many decades, fluoride availability and its usage have increased with the introduction of various alternative modalities. In addition to fluoridated community drinking water, children and adults can readily obtain fluoride from professionally-applied products, fluoride containing toothpastes and mouthrinses, dietary fluoride supplements and self-applied fluoride gels. Among many usages of fluoride water, fluoridation is known to be the most efficient and inexpensive way of controlling dental decay in populations[5-7].

The protective effect of fluoride against dental decay is usually attributed to its capacity to influence enamel formation in such a way that the enamel surface becomes more resistant to acid dissolution. This result is believed to be accomplished by the formation of hydroxy-fluoroapatite in the surface enamel. The incorporation of fluoride, therefore has become the key to the preventive dentistry[8]. Also it is known that the maximum protective effect of fluoride is seen among persons who have been continually exposed to fluoridated water from prenatal. Nevertheless, the controversy over fluoride supplement has caused some people to question the need to administer prenatal fluo-

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rides. The debate over the usefulness of prenatal fluorides began in the mid-1930s with the discovery of mottled enamel of deciduous teeth[9,10]. Prenatal fluoride actively promotes caries resistance in the offspring and is related to the use of supplements[11,12]. Placental transfer of fluoride has been induced by the demonstration of the influence of fluoride on the mineralization of teeth and on their resistance to dental caries[13,14]. Nevertheless, placental transfer of fluoride has been the subject of considerable research and controversy. Although there is no doubt that fluoride passes the placental barrier and is taken up in fetal tissues of many different species, the literatures concerning transfer and fetal-maternal metabolism contains conflicting information[15,16].

Access to fluoride by placental transfer has attracted attention whether systemic fluoride in the pregnant woman can be beneficial to the developing teeth of the fetus. It was estimated that the optimal daily fluoride supplement in pregnancy to be 4 mg/*l* per day[12]. Gardner et al.[17] and Feltman and Kosel[18] first investigated fluoride concentrations in both maternal and fetal serum when the mother had received additional fluoride either in fluoridated drinking water or in fluoride tablets. Their results indicate that some fluoride had accumulated in the teeth and had presumably crossed the placenta. Speirs[19] reported that fluoride simply diffused into the placenta and then diffused passively to the fetus to be incorporated into its skeletal tissues. They suggested that fluoride could be transported through the placenta to the fetus, although the placenta might also function as a partial barrier. Several studies[20-22] have reported that placental transfer of fluoride in human was passive when fluoride intake was low and the fluoride content of tooth germs and mandibular bone increased in relation to the fluoride concentration in drinking water. The placenta possibly serving as a barrier is considered by some to have a special influence on the supply of fluoride to the fetus. Schrotenboer[23] reported that the high concentrations of fluoride in the placenta and speculated fluoride storage and/or diffusion barrier, and the placenta played only a passive role, while others concluded that its role was active, it being either a filter or a concentrator. However, a comprehensive review of Stookey's[24] concluded that there were no meaningful benefits to both the deciduous and the first permanent molars from maternal ingestion of fluoridated water. Leverett et al.[25] reported that prenatal exposure to fluorides in drinking water had little influence on the caries' susceptibility of deciduous tooth. Lewis et al.[26] also found that prenatal exposure to optimally fluoridated water had only a negligible effect in deciduous tooth of children who also consumed fluoridated water postnatally. To obtain maximal cariostasis, it does not necessarily require fluoride supplement throughout the prenatal period of teeth formation. The free passage of fluoride incomplete stage of enamel formation at birth help to explain no benefits

have been observed from maternal ingestion of fluoridated water. The scarcity of reports on fluorosed changes in the deciduous dentition had, in the past, been ascribed to a partial placental barrier. Recently it was known that there was no placental barrier and the phenomenon was thought to be caused by the rapid maternal renal excretion and skeletal uptake of the fluoride. The fetal teeth did not receive the full share of fluoride from the mother. It was commonly believed that the placental acts as a partial barrier to fluoride in order to protect the fetus from toxic amounts.

The purpose of this study was to determine concentrations of the calcium, magnesium, phosphorous and fluoride in fetal from whose mothers had received various levels of fluoride during the pregnancy.

2. MATERIALS AND METHODS

2.1. Animal Control

Twelve adult female and male Sprague-Dawley rats were obtained from Taehan experimental animal center(Breeding and Research center, Korea). Neither females nor males had been used previously for breeding. Twelve 8 weeks old female rats with initial average weight of 180~200 gm, and 10 weeks old male rats with initial average weight of 301~330 gm, were randomly divided into one control group and three experimental groups. The control group was given redistilled water. The experimental groups were given redistilled water containing 1, 5 and 20 ppm fluoride in the form of sodium fluoride. All rats were provided with a standard laboratory chow diet and drinking water ad libitum for approximately 4 weeks(3 weeks for mating, 1 week for breeding). The temperature and humidity in the climate-controlled room, which had a 12 hour light/dark cycle, were 20±2°C and 40~80%, respectively. Animals were housed in separate cages in a room used exclusively for this study. The rats were allowed free access to water and food during the first week of acclimatization to their new environment to the end of experiment. The female rats were housed in separate cages and the male rats were introduced to each cage and left there for 3 weeks. The rats could be considered to have been successfully mated. The 12 pregnant rats were separated a few days before breeding.

2.2. Preparation of Specimens

Immediately after the birth, fetuses were sacrificed and stored in the plastic tube at -85°C(Deepfreezer, Sanyo©, Japan) for further processing. The samples were melted at room temperature for an hour. All fetuses were ashed in a thermo-control furnace(Type 212, Mortia©, Japan) at 600 for 7 hours in porcelain crucibles.

2.3. Determination of Inorganic Substance

All the samples were analyzed the ash content of fluoride, calcium, magnesium and

phosphorus. Nitirc acid(Osaka Chemical©, Osaka, Japan) was added in a ratio of 1 ml acid to 0.1 gm of ash weight. Teflon Cup (Parr Instrument©, Moline, Illnois, U.S.A.) were sealed with a Teflon lid and the samples were put into Acid digestion bomb(Parr Instrument©, Moline, Illnois, U.S.A.). The bomb was transferred to an dry oven and the samples were then digested at 180°C for 2 hours. The levels of calcium, phosphorus and magnesium ions were measured using the Induced Coupled Plasma-Atomic Emission Spectrometer(Jobin Yvon-38S, France).

2.4. Determination of Fluoride

100 ppmF of standard solution was serially diluted within the measurable range. Fluoride standard curves were prepared by diluting the above fluoride standard solution to obtain sodium fluoride solutions of desired strengths, covering a range from 0.001 to 10 mgF/l〈Fig. 1〉. The digested samples were mixed with both 50% sodium acetate and TISAB II buffer, which adjusted the pH to 5.0. The fluoride measurements were made on this solution using a specific ion electrode(96-09, Orion Research Inc., Cambridge, U.S.A.) combined with a reference electrode. The specific electrode was connected to an expandable ion analyser EA 940(Orion Research Inc., Cambridge, U.S.A.), stirring the sample with a magnetic stirrer. Millivolt reading of sample solutions were recorded after waiting for a stable reading. Fluoride concentration of samples were determined from the calibration curve(linear logarithmic equation). Analysis was performed three times for each sample.

2.5. Statistical Analysis

Data analysis was conducted using SAS 8.01(SAS Institute Inc., Cary, NC, U.S.A.).

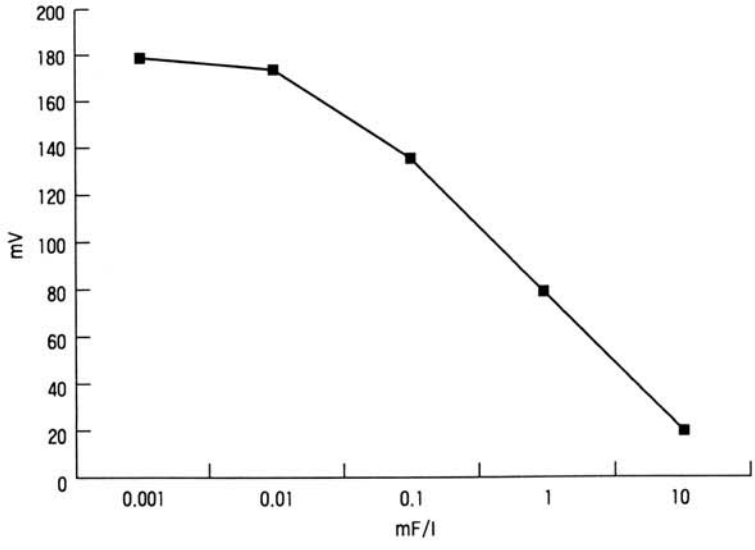

Fig. 1. A typical calibration curve of a fluoride electrode.

Mean ash weight and contents of inorganic substances were compared by the one-way ANOVA. Scheffe's method was considered for post-hoc test. Corresponding p-values were considered significant at values less than 0.05.

3. RESULTS

3.1. The Reproduction Records of Rats

〈Table 1〉 shows the result of the mean number of litters born and the total number of pups. The mean number of litters born were 12.75±3.20 in control, 10.67±6.11 in 1 ppm, 12.33±2.51 in 5 ppm and 10.67±4.50 in 20 ppm. There were no remarkable changes of reproductive rate in rats among any groups. The total number of rats born were 36 in control, 32 in 1 ppm group, 37 in 5 ppm group and 32 in 20 ppm group, respectively. Fluoride concentration used within this experiment could not affect on reproduction rate of rat($p>0.05$).

3.2. Ash Weight

〈Table 2〉 shows the results of mean ash weight in the control and the experimental groups. The maximum ash weight was 107.61 gm and minimum of 96.46 gm. It was the highest in 1 ppm group and lowest in the control group. The ash weight values were 96.46±4.33 gm, 107.61±7.68 gm, 100.02 ±6.73 gm and 103.55±13.64 gm from control to 20 ppm group, respectively. However, there were no significant differences in the mean ash weight among the groups($p>0.05$).

Table 1. The reproduction records of rats receiving different concentration of fluoride in the drinking water

Group	Total numbers of rats born	Means numbers litters born
Control	36	12(12.75±3.20)
1 ppm	32	10(10.67±6.11)
5 ppm	37	12(12.33±2.51)
20 ppm	32	10(10.67±4.50)

Values are mean SD
No statistically significant differences by the one-way ANOVA at level 0.05($F=0.23$)

3.3. Quantitative Analysis of Inorganic Substance in Total Carcass Pups

〈Table 3〉 shows that the contents of calcium, magnesium and phosphorous. The mean calcium levels were 185.37±18.32 ppm in control, 191.04±18.42 ppm in 1 ppm group, 192.19±17.05 ppm in 5 ppm group and 197.87±17.13 ppm in 20 ppm group, respectively. Although the contents of calcium were increased as the fluoride concentration were increased, we found no significant dif-

Table 2. Ash weight of total carcass of pups

Group	Ash weight(gm)
Control	96.46± 4.33
1 ppm	107.61± 7.68
5 ppm	100.02± 6.73
20 ppm	103.55±13.64

Values are mean SD
No statistically significant differences by the one-way ANOVA at level 0.05($F=6.00$)

ferences among the groups($p>0.05$). The mean magnesium levels were 13.49±1.07 ppm in control, 12.56±0.89 ppm in 1 ppm group, 13.84±1.30 ppm in 5 ppm group and 13.93±1.17 ppm in 20 ppm group respectively. The mean phosphorous levels were 196.22±15.43 ppm in control, 190.07±15.69 ppm in 1 ppm group, 202.19±16.16 ppm in 5 ppm group and 206.71±17.84 ppm in 20 ppm group respectively. There were no significant differences between magnesium and phosphorous content among the groups($p>0.05$). The calcium/phosphorous ratios of total carcass pups are shown in table 3. The ca : p ratio of 1 ppm group was the highest. There were no statistical significant differences among four groups($p>0.05$).

3.4. Quantitative Analysis of Fluoride in Total Carcass Pups

〈Table 4〉 showed the contents of fluoride in each experimental group. The mean fluoride levels were 5.74±4.05 μg/g, 32.79±11.72 μg/g, 18.40±11.92 μg/g and 9.11±5.50 μg/g from control to 20 ppm group, respectively. The mean fluoride level of 1 ppm group was significantly higher than that of control group, but the concentrations of fluoride in total carcass pups of 5 and 20 ppm groups were significantly less than that of 1 ppm group($p<0.05$).

Table 4. Contents of fluoride in total carcass pups after administration of sodium fluoride(μg/g)

Group \ Ion	F	
Control	5.74± 4.05	A
1 ppm	32.79±11.72	B
5 ppm	18.40±11.92	C
20 ppm	9.11± 5.50	C

All values are mean S.D.
Statistically significant differences by the one-way ANOVA at level 0.05($F=18.75$).
A, B, C: the same characters are not significant by Scheffe's method comparison at the 0.05 level.

4. DISCUSSION

The contents of calcium, magnesium and phosphorus in the total carcass were increased with the administrated fluoride concentration were increased, but there were no statistical significant differences among groups. The present data was similar with

Table 3. Contents of inorganic substance in total carcass pups after administration of sodium fluoride(× 10^3 ppm)

Group \ Ion	Ca	Mg	P	Ca/P
Control	185.37±18.32	13.49±1.07	196.22±15.43	0.94±0.03
1 ppm	191.04±18.42	12.56±0.89	190.07±15.69	1.00±0.04
5 ppm	192.19±17.05	13.84±1.30	202.19±16.16	0.95±0.06
20 ppm	197.87±17.13	13.93±1.17	206.71±17.84	0.96±0.04

Values are mean SD
No statistically significant differences by the one-way ANOVA at level 0.05($F=0.80$, 2.59, 1.75 and 2.68)

those findings of Montherrat et al.[20], Chen and Whitfold[27] and Babeaux and Zipkin[28].

The present results indicated that the fluoride concentration in 1 ppm group was considerably increased compared that of the control. In those of 5 and 20 ppm groups, the concentrations of fluoride were slightly higher than that of control group but notably lower than that of 1 ppm group. We found that there was the most remarkable increasement in the 1 ppm group. It suggested that the placenta played an active role in accumulation of fluoride. Our result was similar with the findings by Babeaux and Zipkin[28]. They investigated the contents of fluoride of 1 ppm group in the drinking water was about 1.6 times higher than that of 0 ppm group. Also, it was the same results from the previous experiment. Kim and Song[29] showed that the volume of retained fluoride of carcass pups were increased when the mother received fluoride supplement during pregnancy. All these findings support the conclusion that a placental fluoride transfer in fetus when the mother receive fluoride supplement during pregnancy. Gedalia et al.[30] analysed fluoride concentrations in bones 4 to 9 months from a moderate to low fluoride area. They showed a rise in fluoride concentration in the fetal bones in the moderate fluoride area, and a detectable, but smaller and less consistent rise in the low fluoride area, indicating fluoride transfer to the fetus. Also, when the fluoride uptake was low, fluoride passed freely through the placenta, when the fluoride uptake was high, the placenta played a regulatory role, which protected the fetus from excess fluoride. Watanabe[31] analyzed the fluoride content of rat's bone and reported that fluoride was passively transported from the maternal to the fetal body by simple diffusion through the placenta. Other experiments have also demonstrated passive diffusion of fluoride across the placenta[32,33]. Therefore, we estimated that fluoride can be transported through the placenta to the fetus although the placenta may also function as a partial barrier. Above findings might explain that a fluoride placental transfer was possible and that only a small amounts of the offered fluoride passed the placental tissue. This seemed to be consistent with our results. Although, it is well known fact that fluoride does pass the placenta, only less than 1% of the fluoride administered to rat in the drinking water during pregnancy can be transferred to the offspring. Therefore, the rat would require a fluoride intake of over 1 ppm in the drinking water during pregnancy in order to affect the total fluoride content of the pups. We guess that the placenta exerts a partial blocking effect that allows the passage of only limited amounts of fluoride given to the pregnant mother. Based on the present results and the studies conducted over the years, it is now quite well established that fluoride crosses the placenta and can be distributed to the fetus. We may point out that the placenta is a physiological membrane although the amount of fluoride distributed would of course, be dependent

on the dose ingested during a pregnancy.

However, we studied only the effect of prenatal fluoride administration on rats. There should be more intensive studies about the effect of pre and postnatal fluoride administration.

5. SUMMARY

Fluoride has been one of the most widely studied caries-preventive agents. But the effect of prenatal administration had been controversies for many years. The results showed that there were no influence on reproductive rate of rats with administration of fluoride from 0 to 20 ppm during pregnancy($p>0.05$). There was a trend towards slightly increased the mean ash weight in the 1, 5 and 20 ppm groups, as compared with the control group. However, there was no significant differences among groups ($p>0.05$). The contents of calcium, magnesium and phosphorus in the total bone were increased with the administrated fluoride concentration were increased, but there were no statistically significant differences among groups($p>0.05$). The mean fluoride level of 1 ppm group was significantly higher than that of control group, but the concentrations of fluoride in total carcass pups of 5 and 20 ppm groups were significantly less than that of 1 ppm group($p>0.05$).

The results of this study indicate that the amount of fluoride transferred to the offspring, which may produce anticariogenic effects in the primary teeth of their offspring.

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초 록

태생 전 불소투여로 인한 태생직후 백서 골조성 변화

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색인: 불소, 산전투여, 골조성

불소화합물은 치아우식증을 예방하는 데에 가장 효율적으로 이용되고 있는 치아우식 예방제이다. 임신 전 산모의 불소투여로 인한 태아의 치아우식증 효과에 대해서는 다양한 연구들이 보고되고 있지만, 아직까지도 불소의 산전투여가 태아의 골조직에 유용한 가치가 있는지에 대해서는 논란이 많다. 따라서 본 연구의 목적은 태생 전 다양한 농도의 불소를 임신 전후 어미 백서에게 투여 후 이들로부터 태어난 새끼 백서들의 골내의 무기성분들을 분석함으로써 태생 전 투여되는 불소가 새끼 골 형성에 미치는 영향을 관찰하고자 하였다.

생후 8주경의 Sprague-Dawley계 자성 백서 12마리와 10주경의 웅성 백서 12마리를 각각 3마리씩 4개 군으로 분류하여 각각 교미시킨 후, 제 1 군은 대조군으로서 제 3 차 증류수를 투여하였고, 제 2 군은 1 ppm, 제 3 군은 5 ppm, 제 4 군은 20 ppm 투여군으로서 자성 백서가 새끼 백서를 분만할 때까지 무제한 공급하였으며, 어미가 새끼를 출산한 날 즉시 새끼 백서를 수거하였다. 탄화시킨 새끼 백서 시료의 중량을 잰 후, 산분해용기를 사용하여 전처리하고, 각 시료내의 불소는 불소이온 전극 (Orion Res 96 09 BN Combination fluoirde select TVE electrode)을 이용하여 분석하였으며, 칼슘, 마그네슘 및 무기인은 Induced Coupled Plasma-Atomic Emission Spectrometer로 정량분석하여 다음과 같은 결과를 얻었다.

1. 임신기간 동안 0, 1, 5 및 20 ppm까지의 불소투여로 인한 각 군간의 어미 백서의 출생률 및 생식능력에는 통계적으로 유의한 차이가 없었다($p>0.05$).
2. 모든 실험 군간에 새끼 백서들의 평균 탄화중량은 통계적으로 유의한 차이를 나타내지 않았다 ($p>0.05$).
3. 새끼 백서 골내의 칼슘, 마그네슘 및 무기인의 함량은 투여되는 불소의 양이 높아질수록 증가

하는 경향을 나타내었으나, 통계적으로 유의한 차이는 없었다($p>0.05$).

4. 새끼 백서의 골내의 불소 함량은 1 ppm 불소투여군에서 다른 군들에 비해 통계적으로 유의하게 높았다($p<0.05$).

이상의 실험결과로 종합해 볼 때 20 ppm까지의 불소투여는 어미 백서의 생식능력과 출생률에 아무런 영향을 미치지 않았으며, 미량의 불소는 태아에게 전이되어 골 조직내 불소가 축적됨을 알 수 있었다. 또한 20 ppm 정도의 불소투여는 골내의 칼슘, 마그네슘과 무기인의 축적률에 아무런 영향을 미치지 않았다.