BIOCHEMICAL AND HISTOPATHOLOGICAL EFFECTS OF CHRONIC FLUOROSIS ON LUNG TISSUES OF FIRST GENERATION RATS

M. Oncu[1], K. Gülle[1], E. Karaoz[1], F. Gultekin[2], S. Karaoz[3], I. Karakoyun[2], E. Mumcu[4]
Suleyman Demirel University, Faculty of Medicine, Department of Histology and Embryology, Isparta, Turkey[1].
Suleyman Demirel University, Faculty of Medicine, Department of Biochemistry and Clinical Biochemistry, Isparta, Turkey[2]
Kocaeli University, Health High School, Kocaeli, Turkey[3]
Suleyman Demirel University, Faculty of Medicine, Department of Orthopedics, Isparta, Turkey[4]

ABSTRACT
Chronic fluorosis is a slow and progressive process causing symptoms related to several systems particularly musculo-skeletal and dental systems. This study is aimed at investigating the biochemical and histological effects of chronic fluorosis on first generation (F1) rat lung tissues.
Adult Wistar albino rats were used in order to obtain F1 male rats. Female rats were mated with males at a 2:1 ratio. The pregnant rats were given drinking water containing 100 mg/L sodium fluoride during gestation period. The rats had labour at 21± 2 days. During the lactation period the mother rats were given the similar fluoridated water (100 mg/L fluoride). After weaning period, the young animals (1st generation; F1) were given the similar fluoridated water for 4 months time. Then, 9 male rats (F1) were chosen randomly and were sacrificed, and the lungs were removed for biochemical and histological examination. Control group rats were given commercial water containing of 0.07 mg/L fluoride.
In F1 rats, plasma fluoride levels and the levels of thiobarbituric acid reactive substance (TBARS) in the homogenates of lung tissues were found to be increased significantly when compared with the control group. There were markedly histological changes in lung tissues of F1 rats. Alveolar congestion, descuamation of alveolar epithelium, thickened interalveolar septae were observed. Mononuclear cell infiltrations and hyperemic vessels were evident in the parenchymal areas. Moreover, some emphysematous areas were observed. Our biochemical and histopathological results clearly show that chronic fluorosis causes a marked destruction in lung tissues of F1 rats.

Introduction

Fluoride is a potent anion and the most electronegative element as well as being a cumulative toxin (20). Fluoride intake can be either by ingestion or inhalation. Consuming 1 mg fluoride per day is essential for human being (20,1). Fluoride is eliminated almost exclusively via the renal route. In a normal person, urinary excretion of fluoride is approximately 0.1 to 0.5 mg in 24 hours

time. Many investigators observed a good correlation between intake and urinary loss of fluoride (17) .

The clinical picture in fluoride toxicity is seen as a result of accumulated fluoride. Fluoride consumed in high quantities can cause a severe damage in most tissues including primarily dental and skeletal systems. In endemic fluorosis, urinary fluoride excretion has been found to be 1.2 to 10.0 mg per 24 hours (17). Fluoride accumulation in most tissues occurs among residents of areas endemic for high environmental fluoride.

High doses of oral fluoride intake at once results in acute fluorosis of many tissues including stomach, lung, gut, heart, brain, kidney and, neuronal and muscle systems. Additionally, high doses of fluoride has the effects of calcium binding which results in depressed blood calcium levels. It also causes decreased blood oxygen levels and depresses mitochondrial enzyme systems. Elevated potassium levels have also been reported (15, 24, 30, 31, 35).

Chronic fluorosis is a slow and progressive process causing symptoms related to several systems particularly musculo-skeletal and dental systems. However, metabolic, functional and structural damages caused by chronic fluorosis have also been reported in many tissues including kidney, liver, endocrine glands (thyroid, parathyroid and pituitary gland), testis, muscle and neuronal systems (12, 13, 17, 33)

Being highly soluble in water, environmental fluoride is absorbed easily in the stomach and gut. Although high amount of fluoride in plasma is in bound form, a small fraction of it is in ionic form. Fluoride passes easily through cell membranes in its ionic form. Therefore primary involvement of bone and teeth in chronic fluorosis is attributed to the ability of these tissues to accept fluoride ion in exchange for other anions (17).

Isparta City is an endemic area for fluorosis. Residents of Isparta City have been exposed to high fluoride intake by drinking water. This study is aimed at investigating the biochemical and histological effects of chronic fluorosis on first generation rat lung tissues.

Mdterials and Methods

A. Chemical substances and kits: Sodium fluoride (NaF) (Merck, Cat No: 6441), 0.1 M Sodium fluoride Standart (orion Cat. No: 94 04 09). Hayat Danonesa commercial spring water, the chemical analysis of the spring water: Ca^{2+}= 51mg/L, F^{-}= 0.07 mg/L, Mg^{2+}= 9 mg/L, HCO_3^{-}= 179 mg/L, Na^{+}= 2.3 mg/L, SO_4^{-}= 8.2 mg/L, NO_3^{-} = 3.4 mg/L, total = 272 mg/L.

B. Preparation of Drinking Water:

1. 5000 ppm stock sodium fluoride solution: Sodium fluoride of 44.204 g was dissolved in deionized water to result in one liter solution. The stock sodium fluoride solution was kept in a brown colored bottle in refrigerator at +4 °C for a week. This stock solution was prepared weekly. To prepare the water containing 100 mg/L fluoride, 83. 70 ml NaF solution mixed with commercial water (Hayat Danonesa) to result in a 1 liter solution.
2. The commercial water (Hayat DANONESA) given to control group rats was containing 0.07 mg/L fluoride.

C. Animals and treatment

Wistar albino rats were obtained from Suleyman Demirel University Laboratory Supplies (Isparta – Turkey). All animals were maintained in an air-conditioned room with controlled temperature of 24 ± 2 °C. All rats received human care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Sciences and published by the National Institutes of Health.

Adult female rats, aging five months and weighing between 120-140 g were used in order to obtain first generation rats (28). Female rats were mated with males at a 2:1 ratio initially. Females showing sperm in vaginal smear were separated on the day of detection which was considered as day of 0 of gestatio.

The pregnant rats were given drinking water containing 100 mg/L fluoride (NaF) (12,33) during gestation period (28). The rats had labour at 21±2 days. Totally 38 pups were born . During the lactation period the mother rats were given the similar fluoridated water(100 mg/L fluoride). After weaning period, the young animals (1st generation; F1) were given the similar fluoridated water for 4 months time. During all these periods 7 pups died and 14 female and 17 male rats survived. Then 9 male rats (F1) from those 17 rats were chosen randomly and were sacrificed, and the lungs were removed for biochemical and histological examination.

Adult female rats, aging five months and weighing between 120-140 g were used in order to obtain first generation control rats. Four female rats were mated with two males at a 2:1 ratio initially. Two female rats were identified as pregnant by the similar method and were included in this experiment. The pregnant rats were given commercial water containing 0.07 mg/L fluoride . Totally 19 pups were born. During the lactation period the mother rats were given the similar commercial water (containing 0.07 mg/L fluoride). After weaning period, the young animals (1st generation control; CF1) were given the similar commercial water for 4 months time. During all these periods 4 pups were died and 6 female and 9 male rats were survived. Then 9 male rats were sacrificed and the lungs were removed for biochemical and histological examination.

Both F1 and CF1 rats were aging five months and weighing 120-140 g at the end of the experiments.

Biochemical examinations

Before the rats were killed, 6cc blood were obtained intracardiacally for the analysis of fluoride levels. Orion mark ion electrometer and Orion mark (Orion Research, Inc. 500 commings Center, Beverly, MA 0 1915-6199) fluoride selective electrode were used. For biochemical analysis, the left lung of rats were removed and washed with physiological saline. They were then homogenized for 3 min (Ultra-Turrax T25, Staufen, Germany) in cold phosphate buffer in order to provide a 10% homogenate. These homogenates were centrifuged at 6000xg for 10 min to obtain supernatants. The levels thiobarbituric acid reactive substance (TBARS) were determined in the supernatants. Protein content of homogenates was determined by Lowry method (19).

TBARS was estimated by the double-heating method of Draper and Hadley (9) The principle of the method was the spectrophotometric measurement of the colour produced during the reaction to thiobarbituric acid (TBA) with malondialdehyde. For this purpose, 2.5 ml of 100 g/L trichloroacetic acid (TCA) solution was added to 0.5 mL homogenate in a centrifuge tube and placed in a boiling water bath for 15 min. After cooling in tap water, the mixture was centrifuged at 1000xg for 10 min, and 2 mL of the supernatant was added to 1 mL of 6.7 g/L TBA solution in a test tube and placed in a boiling water bath for 15 min. The solution was then cooled in tap water and its absorbance was measured at 532 nm. The concentration of TBARS was calculated by the absorbance coefficient of MDA-TBA complex $1.56x10^5$/cm/M and expressed in nanomoles per gram protein.

Statistical evaluation

For statistical analysis, Mann-Whitney U test was used to compare groups.

Histological examinations

Lung tissues of rats were fixed by immersion fixation method. For immersion fixation, the right lungs were removed from rats and divided into lobes after cleaning. The lobes were fixed in 10% neutral buffered formalin. Two parasagittal sections from each right lung, were processed for paraffin embedding.

Sections were cut at 4-6 mm and stained with hematoxylin-eosin. The slides were then examined by light microscope and photographed. All histological evaluations were made twice under blind conditions (without knowledge of the treatment).

Resulr and Discussion

Biochemical findings

The fluoride levels in plasma were found to be increased significantly ($p < 0.001$) in F1 rats when compared with the control group (**Table 1**).

The TBARS levels were also found to be increased significantly ($p<0.05$) in F1 rats when compared with the control group (**Tabl 2**).

Histological findings

In control group rats the histological appearance of lung tissues was normal (**Fig 1**).

There were considerably histopathological changes in lung tissues of F1 rats, as described below. Alveolar congestion, descuamation of alveolar epithelium, thickened interalveolar septae were observed. There were increased connective tissue mass in the parenchyma of lung tissues. Some emphysematous areas were also observed. There were markedly intraparenchymal mononuclear cell infiltrations (such as lymphocytes, plasmocytes and macrophages) and hyperemic vessels (**Fig 2**).

This is the first study investigating the biochemical and histological effects of chronic fluorosis on the first generation rat lung tissue. The results of the present experiment show that chronic fluorosis caused marked-

TABLE 1

Plasma fluoride levels (mean ± SD, n=9 for all groups).

Group	Fluoride (ppm)
Control	0.04 ± 0.01^{a}
F1	0.13 ± 0.01^{b}

[a,b]: $p<0.001$, Mann-Whitney U test.

TABLE 2

TBARS levels (mean ± SD, n=9 for all groups)

Group	TBARS (nmol/mg protein)
Control	3.76 ± 1.08^{a}
F1	4.98 ± 1.47^{b}

[a,b]: $p<0.05$, Mann-Whitney U test.

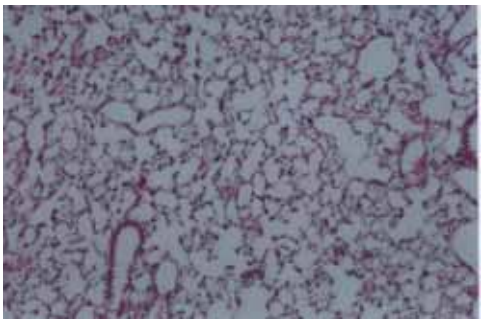

Fig. 1. Control group. Histological appearance of lung tissue is normal.

ly increased lipid peroxidation in lung tissues of F1 rats, resulting in the increased

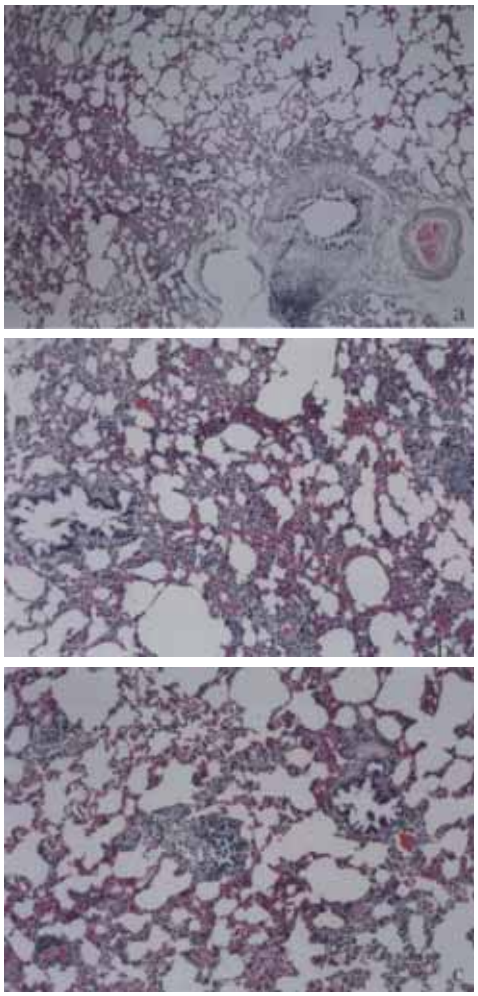

Fig. 2. Alveolar congestion, descuamation of alveolar epithelium, thickened interalveolar septae are seen. Increased connective tissue mass in the parenchyma of lung tissues is present. Some emphysematous areas are also seen. Marke.

TBARS levels.

Previously it had been reported that chronic fluorosis caused alveolar hemorrhage, congestion, oedema fluid, necrosis of alveolar epithelium distortion of alveolar architecture and bronchiolitis in the lung tissues of rats (23, 25). In the present study, in F1 rats, alveolar congestion, descuamation of alveolar epithelium, thickened interalveolar septae were observed. There were incresed connective tissue mass in the parenchyma of lung tissue. Intraparenchymal mononuclear cell infiltrations and hyperemic vessels were evident. Some emphysematous areas were also observed.

A higher fluoride content in plasma and urine of pregnant women living in endemic areas were indicated by Teotia et al (29) and Freni et al (11). reported that women exposed to high fluoride concentrations in drinking water showed decreased birth rates. It has been stated that oral administration of sodium fluoride between 6 – 19th days of gestation caused a significant reduction in uterine weight and the number of implantation (14, 32). Chan et al (4). reported higher levels of fluoride in milk than in maternal plasma. However, Collins et al (18) suggested that sodium fluoride up to 250 ppm did not affect reproduction in rats. Previously it has been reported that vitamin C and D significantly reduced the severity and incidence of fluoride-induced embryotoxicity in rats (14, 32). There is evidence that pathogenesis of tissue damage in fluorosis has been related to oxidative stress and modifications of lipid component of cell membrane (13, 17, 28, 30, 34). In some of these studies, TBARS, an indicator of lipid peroxidation has been found to be increased (13, 34).

Previously it has been reported that high doses of fluoride decreases membrane lipids, especially phospholipids (18, 26, 33). It has been considered that lipid peroxidation caused by free radicals that attacked cell membrane accounted for the decrease mentioned about. Free radicals effect important cellular components such as lipids, proteins,

DNA and carbohydrates. They have a great affinity to macromolecules such as phospholipids, glycolipids, unsaturated lipid acids of glycerides and membrane proteins (5, 7). In addition, several cell functions or components such as enzyme activities, receptors, transmitters, ion channels and permeability could be affected. Although almost all biomolecules are affected by free radicals, lipids are much more sensitive (1, 2, 22). Free radicals are produced by the reduction of molecular oxygen through normal metabolism steps (36). Free radicals are formed continuosly in the human body. Most of them have physiological functions. However, they may be toxic because of extreme formations or being in an environment with inappropriate conditions. This toxicity increases in the context of transition metals such as Fe and Cu (Ciriolo et al. 1991; Slater, 1989).

Some studies have indicated that superoxide radicals can inhibit glutathione peroxidase (3) (GSH-Px) and catalase (CAT) activities (16), and singlet oxygen and peroxyl radicals can inhibit superoxide dismutase (SOD) and CAT activities (10), resulting in an increase in the levels of TBARS.

In the present study, the TBARS levels were found to be increased significantly ($p<0.05$) in the F1 rats when compared with the control group. We consider that large amounts of superoxide radicals have been formed during the metabolism of fluoride and that they have inhibited SOD, GSH-Px and CAT. Biochemical findings were supported by histological observations. There were markedly histopathological changes in the lung tissues of F1 rats.

Fluoride levels in plasma were found to be increased significantly ($p<0.001$) in F1 rats when compared with the control group. In conclusion, in this study chronic fluorosis has been performed experimentally in the first generation rats. Our biochemical and histopathological results clearly show that chronic fluorosis causes a marked destruction in lung tissues of F1 rats.

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