

Effect of high-dose fluoride on antioxidant enzyme activities of amniotic fluid in rats

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Abstract

Objective: To investigate the effect of high-dose fluoride on antioxidant enzyme activities of amniotic fluid and fluoride of serum in rats.

Methods: The experimental study was conducted from January 8, 2008, to December 14, 2010, at the Suleyman Demirel University Experimental Animals Laboratory and the Medical Biochemistry Department Research Laboratory, Isparta, Turkey. Impregnated Wistar albino rats were divided into two equal groups. Group I had controls, while Group II rats were exposed to high-dose fluoride. Group I was given drinking water mixed with 0.1 mg/kg/b.w./day of sodium fluoride, while group II was given drinking water mixed with 10 mg/kg/b.w./day of sodium fluoride for 18 days. At the end of 18 days, amniotic fluid and blood samples were collected from control and experimental groups of pregnancy. Superoxide dismutase, glutathione peroxidase, catalase activities and thiobarbituric acid reactive substances as antioxidant enzymes in amniotic fluid and levels of fluoride in serum samples were investigated.

Results: There were 14 rats, with 7(50%) in each group. Foetal weight in group II significantly decreased compared to the control group ($p < 0.05$). Antioxidant enzyme activities in amniotic fluid were significantly higher in group II than group I ($p < 0.05$) although thiobarbituric acid reactive substances in amniotic fluid and serum fluoride levels were significantly lower in group II than group I ($p < 0.05$).

Conclusion: Fluoride that created oxidative stress inhibited lipid peroxidation and apparently increased the antioxidant defence system.

Keywords: Fluoride, Amniotic fluids, Antioxidant enzymes, Lipid peroxidase, Rat. (JPMA 66: 435; 2016)

Introduction

Fluoride is a highly reactive trace element that is never found in the elemental state in nature. Since fluoride is absorbable, the majority of the fluoride is absorbed rapidly and passively from the stomach and intestinal mucosa into the bloodstream.¹ About 96% of the fluoride in the body is found in bones and teeth.²

Fluoridated drinking water is the main source of dietary fluoride intake for human body.³ Keeping the concentration of fluoride in the range of 0.5 to 1.5 mg/L in water has been recommended by the World Health Organisation (WHO).⁴ The optimal level of fluoride intake is not known with certainty. The tolerable upper intake level for fluoride ranges from 1.3 mg/d for children 1 to 3 years to 10 mg/d for children older than 8 years and adults.²

High concentration of fluoride intake over a long period of time may affect the health of humans and animals. The

severity of the damage is directly related to the concentration and duration of exposure. The primary adverse effects associated with chronic excess fluoride intake are dental fluorosis (discolouration and mottling of teeth) and skeletal fluorosis.⁵ The effects of chronic fluoride exposure have also been linked to effects on other tissues and systems, such as blood, brain, kidneys, parathyroid glands, and liver of animals.⁶ Many studies on laboratory animals over a range of fluoride (F) concentrations (0-250 mg/L in drinking water) indicate that adverse reproductive and developmental outcomes occur at high F concentrations, such as abnormal menstruation, increased miscarriages and other pregnancy complications.^{7,8} Epidemiological studies have also shown decreasing total fertility rate with increasing F levels in drinking water.⁹

Various studies have investigated whether oxidative stress (OS) and lipid peroxidation are involved in the pathogenesis of chronic fluorosis. In organisms, fluoride is thought to inhibit the activity of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT).¹⁰ The excessive production of reactive oxygen species (ROS) at the mitochondrial level and altered antioxidant defence systems are considered to play an important role in the toxic effects of fluoride.¹¹

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The present study was planned to investigate the effects of toxic doses of sodium fluoride on serum fluoride, activity levels of SOD, GSH-Px, and CAT, which are amniotic fluid antioxidant enzymes, and thiobarbituric acid reactive substance (TBARS) concentration, which is an indicator of lipid peroxidation.

Materials and Methods

The experimental study was conducted from January 8, 2008, to December 14, 2010, at the Suleyman Demirel University Experimental Animals Laboratory and the Medical Biochemistry Department Research Laboratory, Isparta, Turkey. All experiments were performed on age-matched impregnated Wistar albino rats weighing 250-270 gr. each at baseline. The study was performed in accordance with the Declaration of Helsinki for Human Research. The rats were housed in well-ventilated cages under a mean controlled temperature at $21 \pm 1^\circ\text{C}$ and in a 12-hour luminous/dark cycle.

Before putting them into a cage with fertile males, vaginal smears were taken and oestrus cycles were observed. Females that were in oestrus and pre-oestrus periods were kept in the same cage during the night (between 1700 and 1900 hours). Vaginal smear was taken again from the females that were separated early in the morning. Whether there was sperm or not was observed. Female rats that had sperm in their vaginal smear were accepted as being one half-day pregnant.

Pregnant rats were divided into two equal experimental groups. Group I, the control group, was fed with normal pellet feed and water containing 0.1 mg/kg/b.w./day sodium fluoride (NaF), while Group II, the experimental group, was fed with normal pellet feed and water containing 10 mg/kg/b.w./day NaF for 18 days. At the end of 18th day, the experiment was terminated because it was thought to be preterm births. The animals were anaesthetised with ketamine 90 mg/kg + Xylazine 10 mg/kg by intramuscular (IM) injection prior to the capitation. At the same time, amniotic fluid (1-2 mL) was collected by syringe from the uteri for determination of the amniotic antioxidant enzymes (SOD, GSH-Px, CAT) activities and TBARS level. Before sacrificing the animals, approximately 6-7 mL of blood was drawn from the abdominal aorta. Blood samples were centrifuged at 3000 rpm for 15 min to remove the plasma and buffycoat.

Chemical substances and kits used were Fluoride (Merck, Cat No: 6441); NaF standards, a.1 ppm (0.1 mg/kg/b.w./day) NaF Standard (Orion Cat. No: 94 09 06), b.100 ppm (10 mg/kg/b.w./day) NaF Standard (Orion Cat. No: 94 09 07); total ionic strength adjusting buffer (TISAB II) (Orion Cat. No: 94 09 09); and Hayat Danone SA spring water (Danone SASabancıGidavelececek San.ve Tic. A.S. 80745 4.Levent-Istanbul).

The chemical analysis was: pH=8, $\text{Ca}^{2+} = 51 \text{ mg/L}$, $\text{F}^- = 0.07 \text{ ppm}$, $\text{Mg}^{2+} = 9.0 \text{ mg/L}$, $\text{HCO}_3^- = 179 \text{ mg/L}$, $\text{Na}^+ = 2.3 \text{ mg/L}$, $\text{SO}_4^{2-} = 8.2 \text{ mg/L}$, $\text{NO}_3^- = 3.4 \text{ mg/L}$. Total = 272 mg/L was used.

For the preparation of fluoride water, a stock of 5000 mg/L stock NaF solution was prepared by dissolving 44.204 g of NaF in 1 L of tap water. This stock solution was kept in a brown-coloured bottle in a refrigerator at $+ 4^\circ\text{C}$ for a week. The stock solution was prepared weekly.

For the control group, 0.83 ml of the NaF stock solution was prepared weekly and mixed with Hayat Danone Spring Water to create a one litre solution that had fluoride of 1 mg/L.

For the treatment group, one litre 83.70 ml fluoride solution was mixed with Hayat Danone Spring Water.

The fluoride ion (F^-) in plasma measurement was read on an Orion Model SA combined with a fluoride electrode as pH/MV.

The SOD activity was determined by the method of Williams et al.¹² For SOD activity, xanthine and xanthine oxidase were used to generate superoxide radicals reacting with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyl tetrazoliumchloride (INT) to form a red formazine dye. The SOD activity was then measured at 505nm on a spectrophotometer by the degree of inhibition of the reaction of washed tissue. The results were expressed as U/L amniotic fluid.

The GSH-Px activity was determined using the method of Paglia and Valentine.¹³ This method is based on GSH-Px catalysing the oxidation of glutathione by cumene hydroperoxide in the presence of glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH). The oxidised glutathione is immediately converted to the reduced form, with a concomitant oxidation of NADPH to NADP⁺. The decrease in absorbance at 340nm was measured. Results were given as U/L amniotic fluid.

CAT activity was measured as described by Aebi.¹⁰ The decomposition of hydrogen peroxide was monitored by measuring the decrease in absorbance at 240nm. The CAT activity was determined as k/mL extracts by taking appropriate absorbance readings based on regression analysis and finally expressed as kU/L amniotic fluid.

Lipidperoxidation (LPO) product was estimated by measurement of TBARS using the method of Anderson and Chen.¹⁴ A 3mL aliquot of 1% phosphoric acid (H_3PO_4) and 0.6% thiobarbituric acid (TBA) solution was mixed with homogenates and the mixture was kept in a hot water bath (100°C) for 45 minutes. It was cooled to normal temperature; 4 mL n-butanol was added and vortexed; and absorbance was measured at 532 nm ($\epsilon = 1.56/105 \text{ M}^{-1} \text{ cm}^{-1}$). The results

were expressed as $\mu\text{mol/L}$ amniotic fluid. Spectrophotometric measurements were measured using a spectrophotometer (Schimadzu UV-VIS 1601, Kyoto, Japan).

Statistical analyses were performed using SPSS 20. Comparison of the two groups was done with non-parametric Mann-Whitney U test. The significance level was set at $p < 0.05$. The results were reported as mean \pm standard deviation (SD) values.

Results

There were 14 rats, with 7 (50%) in each group. Changes in the weight of the foetus in the control group was 9.51 \pm 0.64 gm, and two of the 61 (3.2%) foetuses were seen to be dead. Changes in the weight of the foetus in the experimental group was 8.02 \pm 0.62 gm, and 4 of the 57 (7%) foetuses were found to be dead at the end of the experiment. Oral administration of NaF showed a significant decrease in the average foetus bodyweight at the end of study compared to the control group ($p < 0.001$).

The fluoride content in maternal serum increased significantly in the experimental group when compared to the control group ($p < 0.001$) (Table).

TBARS levels in the amniotic fluid in experimental group were significantly increased compared to the control

Table: The levels of serum fluoride, SOD, GSH-Px, CAT and TBARS in Control and Experimental Groups.

Parameters	Control Group	Patient Group	P
Fluoride (ppm)	0.072 0.031	0.488 0.073	$p < 0.01$
SOD (U/L)	117.2 18.3	95.8 8.8	$p < 0.01$
GSH-Px (U/L)	21.85 2.17	19.22 1.79	$p < 0.05$
CAT (kU/L)	10.41 1.90	7.35 2.10	$p < 0.05$
TBARS ($\mu\text{mol/L}$)	1.32 0.11	2.05 0.21	$p < 0.01$

SOD: Superoxide dismutase

GSH-Px: Glutathione peroxidase

CAT: Catalase

TBARS: Thiobarbituric acid reactive substances

group ($p < 0.01$).

In amniotic fluid, the activities of SOD, GSH-Px and CAT were significantly lower in all fluoride-treated groups than in the control group ($p < 0.01$, $p < 0.05$, $p < 0.05$).

Discussion

Excessive intake of fluoride for a prolonged period can produce injurious effects on the teeth, skeleton and soft tissues such as the brain, thyroid, liver, kidney and reproductive organs. In recent years, various authors have

investigated the relationship between fluoride toxicities and fluoride-induced OS in people¹⁵ and animals.^{4,5,16-21}

A study²² compared foetal growth of rats that were given 40ppm fluoride with potable water on the 20th day of pregnancy with a control group and it was seen that there was a significant decrease in body weight and length. In our study, the decreased body weight of foetuses observed in the experimental group compared to the controls also indicated loss of weight due to excessive breakdown of tissue proteins.

In the current study, the serum fluoride level was found to have increased significantly in the study group compared to the control group. These results also show compatibility with an earlier study¹⁹ that showed that high levels of fluoride caused increases in serum amniotic fluid fluoride levels.

Reduced glutathione (GSH) is known to protect the cellular system against the toxic effects of lipid peroxidation as a co-substrate for glutathione peroxidase (GPx) activity.¹⁹ The depletion in the activity of GPx may result in the involvement of deleterious oxidative changes due to the accumulation of oxidative toxic products. SOD is an important defence enzyme, which converts superoxideradicals to hydrogenperoxide.²⁰ CAT decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals.²¹ The reduction in the activity of these enzymes may be due to OS exerted by fluoride in toxication.

Many studies also indicate that fluoride toxicity can induce free radical toxicity in humans and animals.²²⁻²⁴ Fluoride has been demonstrated in vivo and in vitro to cause increased lipid peroxidation in erythrocytes of humans and in blood and tissues of experimental animals.²⁵

In 2004, a study in which experimental rats were orally treated with 25ppm of fluoride/rat/day for 8 and 16 weeks, respectively, revealed an increase in the level of lipid peroxides along with a concomitant decrease in the activities of SOD, CAT, GSH-Px, and reduced glutathione content was observed in high-dose fluorinated groups of rats.³

In our study, a marked increase in the levels of TBARS was observed in amniotic fluid of high-dose fluorinated rats. Increased lipid peroxidation in the amniotic fluid can be due to increased OS in the cells as a result of depletion of the antioxidants scavenger system.

A recent study¹⁷ determined that in rats that were given 50ppm fluoride with potable water for a week, SOD and GSH-Px, which are from plasma antioxidant enzyme system, activity levels decreased and TBARS levels increased significantly.

In our study, the amniotic fluid of rats were used instead of 10

mg/kg/b.w./day. NaF exposed the serum of rats. To the best of our knowledge, ours is the first experimental study on the effects of high-dose NaF on antioxidant enzyme activities and TBARS levels in amniotic fluid of rats. Since high-dose NaF has passed to the placenta, excess amounts of superoxide radical ($O_2^{\cdot-}$) formed in the environment, SOD, GSH-Px and CAT antioxidant enzymes that catalysed superoxide radicals ($O_2^{\cdot-}$) in amniotic fluid were inhibited, and TBARS levels, which show lipid peroxidation increase, rose.

Our study has a limitation. Fluoride crosses the placenta of humans and animals and is absorbed by the foetus.²⁶ Thus, maternal supplementation during pregnancy results in increased fluoride concentrations not only in maternal blood, but also in cord blood and offspring tissues, especially bones and teeth.²⁷ We didn't investigate fluoride levels in amniotic fluid and infant blood because of inadequate equipment.

Conclusion

Oxidative stress induced by fluoride played an important role in the pathogenesis of fluorosis that may result in tissue damage and other secondary complications.

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