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Research Article

Histochemical Shifts In the Profile of Ovarian Protiens and Sudanophilic Lipids of Sexually Mature Cycling Females of Swiss Albino Mice Due To Sodium Fluoride Ingestion

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ABSTRACT

Distribution of protein and sudanophilic lipid, were studied following suitable techniques in ovary of a mice exposed to chronic dose of NaF. NaF was given for 5 days in two doses 5 mg/kg BW and 10 mg/kg BW in drinking water. Unilaminar, bilaminar, multilaminar, Graffian follicles and atretic follicles were observed for possible changes. A decrease trend in protein and sudanophilic lipid, was observed after 5 mg/kg BW as well as 10mg/kg BW NaF. The study deals with histochemical distribution of protein (P) and Sudanophilic lipid in ovary of a Swiss mice after ingestion of 5 mg/and 10 mg/kg BW dosage of sodium fluoride in drinking water. Histochemical reactivities of protein and sudanophillic lipid were evaluated in primary, secondary, tertiary, graffian and atretic follicles. Significant changes were observed in uni-, bi- and multilaminar follicles. Interestingly a decreasing trend in staining intensity was observed after ingestion of 5 mg/kg BW and a further decrease after ingestion of 10 mg/kg BW dosage of NaF was recorded. Changes in protein might be related to either decrease in reserve protein or interference in cellular synthesis by fluoride. Decrease in lipid might be related to release of steroid hormone.

Key Words: Fluoride, Toxicity, Ovary, Proteins, Lipids, Albino Mice

INTRODUCTION

Two principle functions of mammalian ovary are considered to be exocrine activities (folliculogenesis and ovulation) and endocrine secretions (estrogens and progesterone) which are under the regulatory control of hypothalamo-adenohypophysial hormones (Singh and Krishna, 1997). While these two activities occur continuously in some on attainment of puberty, in others they either occur annually, biannually or several times in a year. Cell division, growth maturation and secretory activities take place during folliculogenesis/oogenesis in the ovarian cells and therefore tissues require a sustained turnover of precursors and the mobilization of protein, lipid and glycogen which subserve variety of functions. They are important constituents of the cell membrane and the organelles and play a critical role by acting as receptors, enzymes, conjugates, precursors; and as substrates for supplicating energy to the intricate events that occur in the ovary (Jaroli and Lall, 1987; Bano *et al.*, 1996.). Site and pattern of protein and lipid distribution in ovarian tissues has been studied in several mammalian species including rat, guinea pig, bat (Guraya, 1973a, Guraya, 1973b, Guraya, 1973c; Jaroli, 1980). However, little is reported in mice. One of the most popular methods to determine whether fluoride causes lipid peroxidation and to what extent it does so has been to determine MDA levels and antioxidant enzyme activities in erythrocytes. The erythrocyte cell membrane is known to be highly sensitive to free radical oxidation due to its unsaturated fatty acid content. There is no report in the literature on the effect of sodium fluoride ingestion on distribution of protein and lipid in the ovary of albino mice. The present study was attempted to histochemically localize protein and lipid and to understand the shifts that occur in these constituents after sodium fluoride exposure in the ovarian cells/layers and the mechanism of these shifts in the ovary of adult female mice used as a model for the study.

MATERIAL AND METHODS

Dose Schedule

Sexually mature, healthy, female Swiss albino mice of Wistar Strain were bred in the laboratory (B.W. 20.5 q 5 gm) and were used as the model for this study. They were given sodium fluoride in two different doses - 5 mgs/kg B.W. (Group I) and 10 mgs/kg B.W. (Group II) in drinking water for 5 days. Ovaries were excised surgically under semi-sterile conditions, freed of connective tissue and blood clots, fixed in chilled 10

Ovaries were excised surgically under semi-sterile conditions, freed of connective tissue and blood clots, fixed in chilled 10 per cent neutral formalin at 4 degree centigrade, for 18 hrs. 8micrometers thick cryosections were cut and used for the study.Sexually mature, Swiss albino mice (BW 25gms) were bred in our zoology lab. for experimental purpose. The contralateral ovaries of each female was excised and weighed separately and processed as follows:

Histochemical study

Protein: For staining of protein, the left and right ovary were taken out from a cervically dislocated animal and fixed in aqueous Bouin for 16-18 hrs. They were dehydrated in graded ethanol series, cleared in xylene, infiltrated with wax and embedded in paraffin wax. 5 micrometer thick serial sections were cut on cryotome. Sections were stained with mercuro bromophenol stain as described in Pearse (1968). Every alternate section was visually appraised and graded for determining intensity of protein staining in the Oocytes, granulosa cells investing the various follicle types, interstitial tissues, corpus luteum and degenerating follicles. Suitable areas manifesting characteristic protein staining were microphotographed and observed.

Sudanophilic lipids: Contralateral ovaries were fixed in calcium formol for 6-10 hours. They were postchromated and embedded in gelatin. Frozen sections were cut at 10 micrometers and stained with Sudan IV as per the technique described by Pearse (1968) to detect the histochemical site and pattern of distribution of lipids in the various histological constituents of the ovary and the alterations that occur in them due to NaF ingestion.

RESULTS

Histochemical data

***Protein*:** In the ovary of sexually mature, controlled Swiss Albino mice, protein staining was visualized in oocytes, granulosa cells investing various follicle types, theca externa and interna, interstitial tissues and stroma. This ranged from moderate to high. The granulosa cells of uni and bi-laminar follicles showed slight protein staining. However, in ovaries treated with fluoride (5mg and 10mg/kg bw)as shown in Fig:1-3Many cells undergoing atretic changes manifested moderate reactions. Various degenerating pre-antral follicles also showed weak (+ve) staining in its dissoluting mass of cells (Figs. 1-3).

Sudanophilic lipids: In the ovary of sexually mature healthy Swiss Albino rice, intense lipid activity manifested by Coalesced granules of various sizes were observed in the growing follicles adjacent to ovocytes in the ovarian cortex. Granulosa cells of healthy primary and secondary follicles manifested intense lipid staining, interstitial tissues. In experimental females, several antral follicles observed in various stages of atresia displayed (+ve) lipid activity. Degenerating granulosa cells and ovum in dissolution state were also observed to be Sudanophilic (Figs. 4-6).

DISCUSSION

The present study highlights the histochemical comparison of the site and paradigm of mercuro-bromophenol positive proteins, in the various ovarian cell types in ovary of a female mice challenged by two doses 5 mg and 10 mg/ kg BW of sodium fluoride for five days. The pattern of distribution of mercurobromophenol positive proteins in response to the dose of NaF does not exhibit marked alterations. The granulosa cells of unilaminar or bilaminar antral follicles showed a decremental staining in mice after NaF administration. This was evident at 5mg/kg treatment dose and subsequently, changes in protein staining in the uni-, bi-, and multilaminar follicles were differential in response to 10 mg dose of NaF. The graffian follicles and lutein cells of corpus luteum also exhibited significant decrease in the protein staining after NaF treatment. These results clearly demonstrate that NaF causes considerable changes in the cellular reserves of protein which may be interpreted as either decline in protein reserve or decreased caspase and BCl2 protein synthesis are produced during folliculogenesis which are involved with progressive changes experienced by growing healthy follicles. This observation correlate well with our observations and therefore it may be suggested that decline in caspase and BCl2 protein reserve may cause disturbances in the follicular development. **(**Hussein, 2005; Erickson and Shimasaki, 2001). Certain proteins are known to be synthesized under the inductive influence of estrogens. Such proteins are designated as the "Marker of estrogens "surge11. Our observation is substantiated by the observation of (Soy and Muscle, 2004) who also observed the decrease in protein concentration in somatic tissues after fluoride ingestion.

Present study also indicates in the various NaF treated groups, the "turn over" of stage specific proteins which is altered. Thus, decremental trend observed may be due to lowered kinetics of protein synthesis which could be further correlated to a decrease in the RNA transcription.

A strict parallelism between these two processes has not

Figure 1: T.S.Of control ovary stained with Mercurobromophenol Blue to Demonstrate protein. (NOTE intense staining of preovulatory follicle).

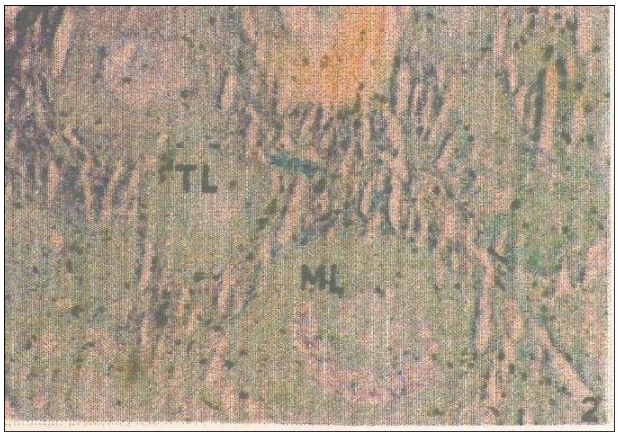

Figure 2: T.S.of an ovary treated with 5mg\kg Bw.NaF.Weak Staining in Tri & multilaminar follicles for proteins.

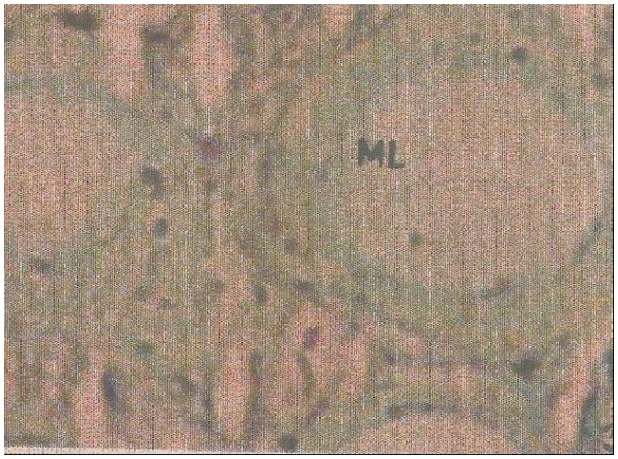

Figure 3: T.S.Of an ovary treated with 10mgs\kg dose of NaF.
Note-Very weak staining for proteins in tri and multi- laminar follicles (X40).

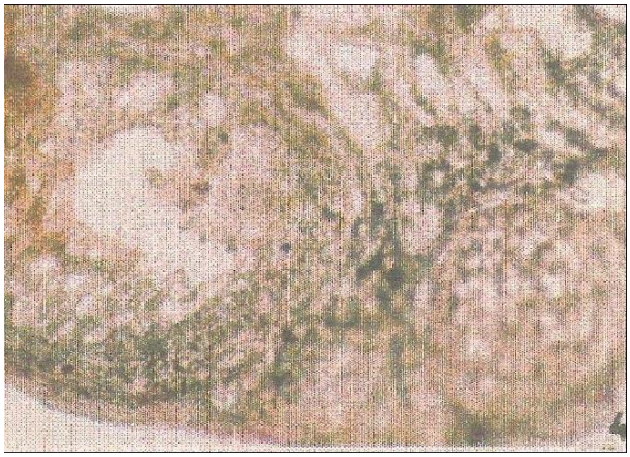

Figure 4: very Weak staining for pseudanophillic Lipid in follicle Cells.(X40) of ovary from mice treated with 10mgs\kgBWt

Figure 5: Weak staining for pseudanophillic Lipid in follicle Cells.(X40) of ovary from mice treated with 5mgs\kgBWt

Figure 6: T.S.of ovary from control mice stained with sudan Black IV for sudanophillic lipids.

Table 1: Showing Intensity of staining of proteins before and after giving NaF doses.

Group	Intensity of staining
][Control	Dark
Test(5mgs dose)	weak
Test(10mgs dose)	Very Weak

TABLE-2: Intensity of staining of Sudanophillic Lipids before and after giving NaF doses.

Group	Intensity of staining
Control	Dark
Test(5mgs dose)	weak
Test(10mgs dose)	Very Weak

been firmly established in the literature. The disturbance in the protein synthesizing system in fluoride treated mice may be also attributable to a decrease in the activity of group of enzymes which catalyze the key processes of cellular metabolism. Al-Hiyasat et.al.(2000) discussed the possibility of fluoride interfering with binding of aminoacylt-RNA to the ribosomal RNA template.This may in turn interfere with the polypeptide synthesis . When compared with controls, both the low- and high fluoride exposed groups revealed altered expression of several proteins related to cognition in both rats and humans, including decreased protein expression for two nicotinic acetylcholine receptors (alpha- 4 and alpha-7 nAChR), increased expression for phospho- and total ERK1/2 and phospho-MEK1/2, and decreased activation rate of phospho-ERK1/2. membrane potential. Inhibition of enzymes involved in cellular metabolism is one of the primary toxic effects of F-. Enzymes in the glycolytic pathway, such as hexokinase, enolase, and pyruvate kinase, are all subject to F- inhibition, as well as antioxidant enzymes such as superoxide dismutase (SOD).Na+/K+-ATPases are also inhibited by F-, leading not only to ATP depletion, but to disturbances in the cell's membrane potential. Studies on enzymatic inhibition caused by F- showed that the inactivation of a large number of enzymes requires concentrations of 10-2 to 10-4 M l-1 (Adamek et al., 2005). Fluoride exposure has also been associated with oxidative stress in soft tissues in animals such as liver, kidney, brain and testes (Guo *et al.,* 2003; 2004;Krechniak and Inkielewicz, 2005). All these affect the sequential changes in the processes of cell growth, cell division and cell differentiation in the germ cells of ovarian epithelium.

Results of this study show that, sublethal dose of 5 mgs/kg B.W. (Group I) and 10 mgs/kg B.W. (Group II) of NaF has deleterious effect on the site and paradigm of distribution of mercurobromophenol positive protein in the ovary of post pubertal cyclic females of albino mice. These changes in protein might be related to either decrease in reserve protein or interference in cellular synthesis by fluoride.These substrate deficiencies would have adverse effects on the role of growth, folliculogenesis, maturation, ovulation and luteinization of granulosa cells.Results of present study also show decreasing trend in sudanophilic lipid in various follicular cell types. Decreased lipid intensity in present study after NaF administration suggests release of steroid hormone due to destructive changes in various follicles.

Little attention has been paid to the, mammalian ovary with particular reference to activities of lysosomal enzymes in ovarian structure and functions. Although, alterations in the structure, functions and metabolism of several soft tissues were studied in both experimental animals and human in relation to different doses of fluoride, experimental evidences about the possible deleterious effects of fluoride in ovary are completely lacking. Therefore, the present study was undertaken to investigate the effects of fluoride on site, pattern and functional relationship of, Proteins and Lipids in the ovary of pubertal Swiss albino mice. . The present study was attempted to histochemically localize protein and lipid and to understand the shifts that occur in these constituents after sodium fluoride exposure in the ovarian cells/layers and the mechanism of these shifts in the ovary of adult female mice used as a model for the study. This study will surely help in the study of similar parameters in humans and other mammals.

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