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EFFECTS OF METHOXYCHLOR ON THE REPRODUCTIVE SYSTEM OF THE ADULT FEMALE MOUSE: 2. ULTRASTRUCTURAL OBSERVATIONS

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Abstract — The purpose of this study is to examine the effects of the pesticide methoxychlor (MXC) on the ultrastructural appearance of the different cellular compartments of the mouse ovary. Sexually mature (7- to 8-week) virgin female CD-1 mice were exposed to 5.0 mg MXC (50% technical grade) via oral gavage for 5 consecutive days each week for 4 weeks. Control groups received either 0.025 mg estradiol-17β (E-17β) or the sesame oil vehicle for the same time period. Twenty-four hours following the final exposure, animals were sacrificed. Ultrastructural observations revealed increased lipid accumulation in interstitial cells and theca cells of both estradiol-treated and 5.0 mg MXC-treated mice. This would suggest that these cells are unable to synthesize and secrete steroids. Thus, this commonly employed pesticide appears to closely mimic those effects on the female ovary induced by estrogen.

Key Words: methoxychlor; ovary; toxicology; ultrastructure.

INTRODUCTION

Methoxychlor (MXC; 1,1,1-trichloro 2,2 bis[*p*-methoxyphenyl]ethane) is a widely used chlorinated hydrocarbon pesticide. It was developed to replace both DDT and chlordecone. Methoxychlor is considered to be less toxic than DDT and chlordecone (1), however, it has been shown to elicit significant effects in the mammalian organism.

The reproductive system has been reported to be a target of MXC at all stages of development. Prenatal exposure of rats to MXC resulted in an increase in the number of resorption sites in the uterus and in the number of anomalous fetuses (2). Prenatal and postnatal exposure to MXC induced precocious vaginal opening in rats (3) and hypertrophied uterine and oviductal epithelium in female mice (4).

The toxic effects of MXC are not limited to the undifferentiated and developing reproductive system. Martinez and Swartz (5) reported a decrease in ovarian weight and an increase in the number of large atretic follicles in ovaries of adult mice exposed to MXC for 4 weeks. This finding is significant in that the fully differentiated female organs of reproduction are susceptible to MXC exposure. The purpose of this study is to examine the ultrastructure of the different cellular compartments of the MXC-exposed adult ovary to ascertain the subcellular toxic effects of MXC and to determine whether such effects are detrimental to the reproductive capacity of the mature female mouse.

MATERIALS AND METHODS

Adult virgin female CD-1 mice (Charles River Breeding Laboratories, Wilmington, MA), 7 weeks old, were used in this study. They were housed in animal quarters with a 12:12 light:dark cycle. Food and water were provided ad libitum. After a 7-day period of acclimatization, mice were randomly distributed into 3 groups containing at least 8 mice each. All groups were treated for 4 weeks. Groups were exposed via oral gavage to either sesame oil, estradiol-17β (E-17β; Sigma Chemical Company, St. Louis, MO) or methoxychlor. E-17β (0.025 mg) and MXC (5.0 mg) were dissolved in sesame oil and administered in a 0.2-mL volume each day of exposure. The dosage of

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MXC is approximately equivalent to 200 mg/kg. This dosage of MXC was selected since it has been shown to result in significant effects on the ovary (5). The MXC, which consisted of 50% MXC, was provided by Dr. V. Eroschenko of the Department of Biology at the University of Idaho, Moscow, Idaho. The group treated with the pharmacologic dose of E-17β served as a positive control in verifying whether the effects observed in MXC-treated animals were due to the estrogenicity of the pesticide or to the inherent toxicity of the chemical itself.

Weekly procedures consisted of 5 consecutive days of exposure to the chemicals, followed by 2 days of no treatment. This timetable was established to mimic an ordinary 5-day work week, representing the maximum weekly exposure to which a human female working with such a compound might be subjected.

Animals were killed by cervical dislocation 24 h after the final exposure. One ovary from each animal of each treatment group was removed and fixed in cold (4 °C) glutaraldehyde for 72 h in preparation for electron microscopy. All animals were in estrus on the day they were killed; however, it was a persistent vaginal estrus in those mice exposed to MXC and E-17β and a normal estrus in sesame oil controls. Following fixation, ovaries were postfixed in 2% osmium tetroxcide in 0.2 M cacodylate buffer (pH 7.2). They were dehydrated and then embedded in Polybed 812/Araldite capsules. The sections obtained were stained with uranyl acetate and lead citrate and observed on a Phillips EM301 electron microscope.

The cellular compartments of the different treated ovaries, including granulosa cells, theca cells, and interstitial cells, were evaluated for ultrastructural morphologic changes. Atresia at the ultrastructural level was assessed according to the criteria established by Gondos (6), which included the presence of disrupted cytoplasmic organelles, chromatin condensation and retraction of microvilli.

RESULTS

Electron microscopic studies revealed that the most dramatic change in the ovary occurred in the interstitial compartment. The interstitial cells in animals treated with 5.0 mg MXC contained large amounts of lipid in their cytoplasm (Figures 1 and 2). Similarly, in animals treated with E-17β, these same cells had an abundance of lipid (Figures 3 and 4). Interstitial cells in sesame oil treated mice, on the other hand, contained minimal amounts of the lipid material (Figure 5). The majority of the lipid bodies found within the 5.0-mg MXC- and the E-17β-treated groups were only slightly osmiophilic, whereas those found in sesame oil controls were highly osmiophilic.

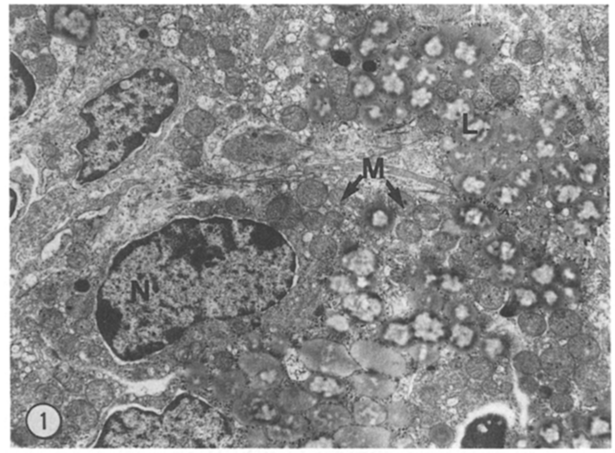

Fig. 1. Interstitial cells of an ovary from an animal treated with 5.0 mg MXC for 4 weeks, showing an increase in the amount of lipid droplets (L) and mitochondria (M) associated with it. Nuclei of these cells can also be observed (N). 4104×

Most of the mitochondria in these cells, in all three groups, were rounded with tubular cristae and appeared healthy. In some animals of the E-17β and 5.0 mg MXC-treated groups the cristae appeared to be more lamellar. A great majority of the mitochondria was found to be associated with lipid in all groups (Figures 2 and 4). The nuclei of these interstitial cells were irregular in shape, with heterochromatin distributed along the nuclear membrane.

Similarly, there was an increase in the accumu-

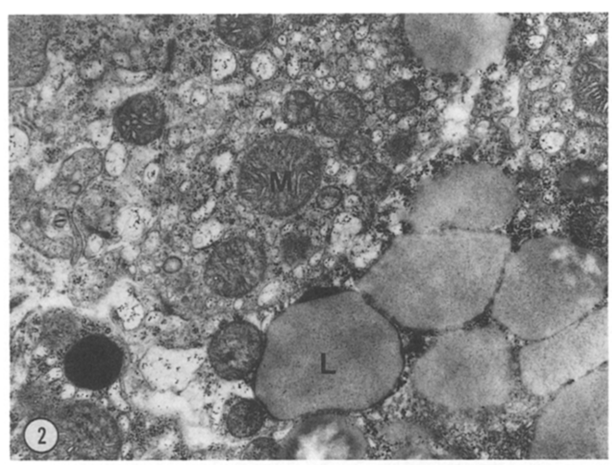

Fig. 2. Interstitial cells of animals treated with 5.0 mg MXC for 4 weeks showing the same condition described above. Mitochondria (M), lipid droplets (L). 10 800×

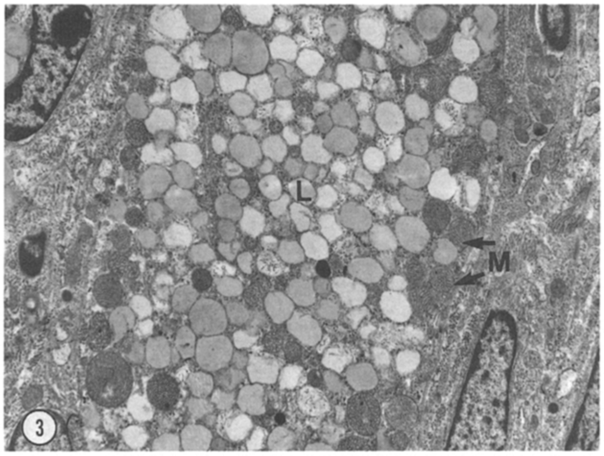

Fig. 3. Interstitial cells of an ovary of an estradiol-treated animal containing large amounts of lipid (L) and associated mitochondria (M) following 4 weeks of exposure. 4104×

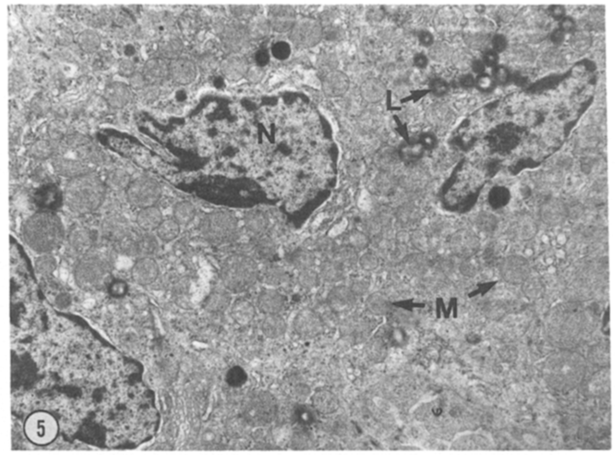

Fig. 5. Interstitial cells of an ovary of an animal treated with sesame oil for 4 weeks, showing small amounts of lipid (L) and large amounts of mitochondria (M). Irregular nuclei (N) of interstitial cells can also be observed. 4104×

lation of lipid and rough endoplasmic reticulum (RER) within the theca cells surrounding both large atretic and large healthy follicles in ovaries of those animals treated with 5.0 mg MXC (Figure 6) and E-17β (Figure 7), compared to those of the sesame oil treated animals (Figure 8). Theca cells in ovaries of mice exposed to E-17β or 5.0 mg MXC for 4 weeks displayed a similar association between the lipid and mitochondria (Figures 9 and 10) as previously seen in interstitial cells. The theca cells in these two groups contained endoplasmic reticulum principally of the rough type with little smooth endoplasmic reticulum (SER) visible (Figure 11), whereas these same cells from sesame oil control animals had ER of both smooth and rough types in approximately equal amounts.

The granulosa cells in healthy and atretic follicles of estrogen and MXC-treated animals did not appear to differ from the controls. These cells in all three groups contained large amounts of free ribosomes and RER. The mitochondria were rounded or elongated with lamellar or tubular cristae. The Golgi apparatus was easy to observe in these cells in all of the groups.

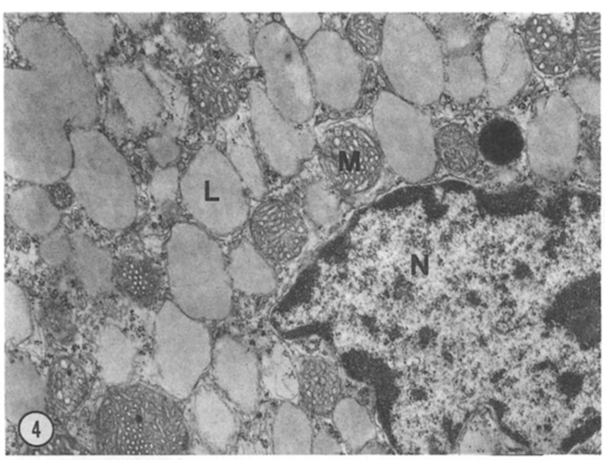

Fig. 4. Mitochondria (M) with tubular cristae associated with lipid (L) in an ovary of an animal treated with E-17β for 4 weeks. A nucleus (N) of an interstitial cell can also be observed. 10 800×

Fig. 6. Thecal cells surrounding a large atretic follicle of a mouse treated with 5.0 mg MXC for 4 weeks, showing large amounts of lipid (L) and elongated nuclei (N) of theca cells. 4104×

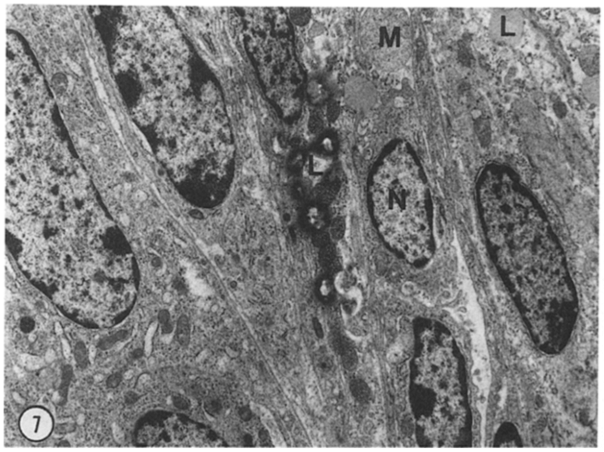

Fig. 7. Thecal cells surrounding a large atretic follicle of an ovary of an animal exposed to E-17β for 4 weeks, showing accumulation of lipid droplets (L). Mitochondria (M) and nuclei (N) of theca cells. 4104×

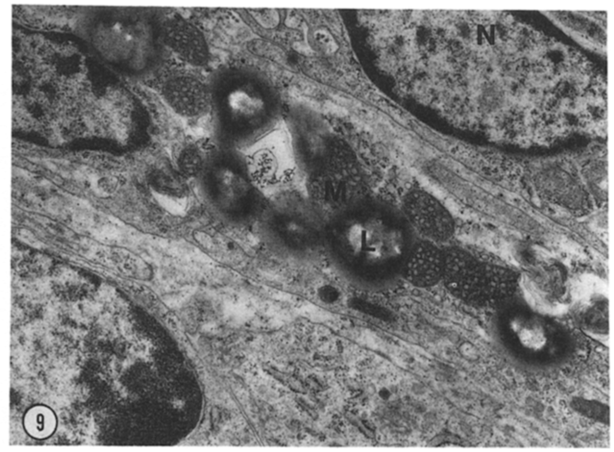

Fig. 9. Detail of the mitochondria (M) in contact with the lipid droplets (L) in a theca cell of an animal treated with E-17β for 4 weeks. The nucleus (N) of a theca cell can also be observed. 10 800×

DISCUSSION

Previous work has shown that 4 weeks of exposure of the adult female mouse to MXC results in a rapid display of persistent vaginal estrus and a significant decrease in ovarian weight along with a significant increase in the percent of atresia in large follicles (5). Thus, the adult ovary appears to be a target organ for the effects of MXC. Very little information is available elucidating the effects of toxic substances on compartments of the ovary at the ultrastructural level. The present investigation presents an ultrastructural evaluation of cellular changes induced within the ovary by MXC. Alterations observed in subcellular organelles can be very subtle indicators of chemical toxicity.

Steroid-secreting cells of the ovary have little granular endoplasmic reticulum (RER) and relatively few ribosomes. They have very extensive smooth endoplasmic reticulum (SER) and tubular or vesicular mitochondria. In addition, lipid droplets are present in these cells. In the present investigation, these steroid-secreting cells in sesame oil treated animals possessed these typical subcellular characteristics.

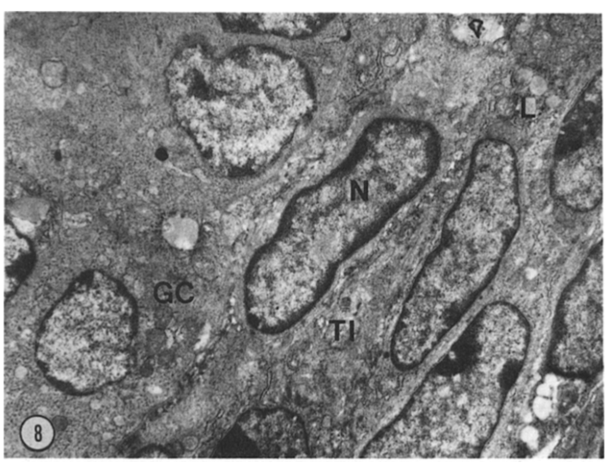

Fig. 8. Thecal cells (TI) surrounding a large atretic follicle in an ovary of an animal treated with sesame oil for 4 weeks. Only minimal amount of lipid (L) can be observed in these cells. Granulosa cells (GC) and basement membrane (arrowheads) can also be observed. Nucleus of a theca cell (N). 4104×

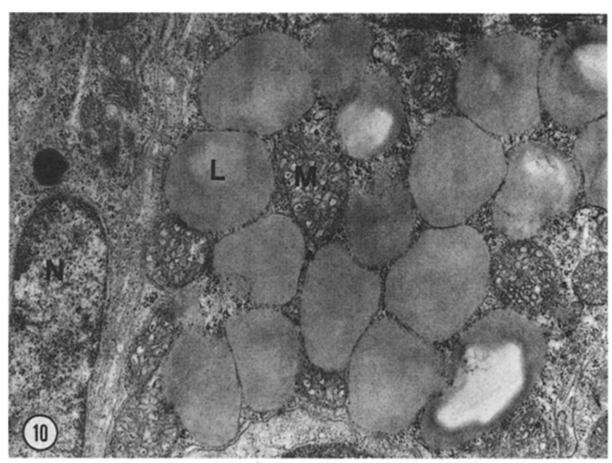

Fig. 10. Close relationship of mitochondria (M) and lipid droplets (L) in the theca cells of an ovary of a 5.0 mg MXC-treated mouse after 4 weeks of exposure. The nucleus (N) of a theca cell can also be observed. 18 000×

Fig. 11. Rough endoplasmic reticulum (r) in a theca cell of an ovary of an animal treated with 5.0 mg MXC for 4 weeks. Lipid droplet (L), nucleus (N). 18 000×

The ultrastructural characteristics of ovarian cells in both estradiol- and MXC-treated mice resembled each other very closely and were quite different from controls. The most remarkable difference was seen in the interstitial cells. The interstitial compartment is capable of producing all classes of steroids, with androgen being the major one (7). Interstitial cells of 5.0 mg MXC- and E-17β-treated mice possessed slightly different characteristics than those of control mice. Whereas the mitochondria were rounded with tubular cristae, the endoplasmic reticulum was principally of the rough variety. Lastly, but most importantly, there was lipid present as one would expect in such cells, but the amount of lipid far surpassed that found in cells from control animals. Similar increased lipid content in interstitial cells was reported by Haney and colleagues (8) in adult mice exposed prenatally to diethylstilbestrol, a synthetic estrogen.

Theca cells are another source of steroids within the ovary. Theca cells surrounding large follicles of 5.0 mg MXC- and E-17β-treated mice were expected to exhibit large amounts of SER since these cells produce much of the steroids, but they were found to contain a large amount of RER, free ribosomes, and minimal SER.

The fact that there is a much-reduced amount of SER in the theca cells of MXC-treated mice compared to what one would expect to see in steroid-secreting cells and that there is a large accumulation of lipid in both theca and interstitial cells may indicate that these cells are not presently involved in steroidogenesis. Brook and Clarke (9) reported a similar ultrastructural appearance in the interstitial cell compartment of the wood mouse and suggested that the small amount of SER and the large amount of lipid indicated that these cells may be relatively inactive. This same designation of "inactive" was also given to lipid-rich luteal cells in rats shortly after hypophysectomy (10). These lipid droplets are thought to comprise a pool of cholesterol or other steroid precursor (11).

It has been theorized that the high accumulation of lipid in such tissues as a corpus luteum indicates a minimal level of steroid release (12,13). It is possible that both exogenous estrogen and MXC alter the steroidogenic process, with a resultant increase in lipid accumulation. These cells apparently retain their ability to synthesize lipids, but lose their capacity to convert the lipids into steroid hormones (14). The fact that this increased accumulation of lipid is seen in theca cells surrounding both atretic and healthy follicles might indicate that the increased lipid content in the theca cells seen at the ultrastructural level might be an early indicator of atresia that is not apparent at the light microscopical level. Bagwell and colleagues (15) reported that an increase in lipid content in corpora lutea of hysterectomized guinea pigs following prostaglandin treatment was a prelude to degeneration of these cells. Exactly how the interference in steroidogenesis induced by estrogen and MXC occurs is not known.

As is well known, application of steroids causes a feedback inhibition of pituitary hormone secretion (11), causing a lack of stimulation in ovarian cells to produce steroids. This appears to be the situation present in animals treated with the steroid E-17β. This same situation can be expected in animals treated with MXC, since this substance has estrogenic activity. Evidence of this is also found in the fact that administering gonadotropins to steroid-producing cells causes a marked decrease in the number and size of lipid droplets (16), which is exactly the opposite situation of the one observed in E-17β- and MXC-treated ovaries.

The granulosa cells in large atretic and healthy follicles in ovaries of animals treated with E-17β and 5.0 mg MXC contained large amounts of RER, mitochondria with lamellar or tubular cristae, and an abundance of free ribosomes, which are characteristic of normal granulosa cells (17,18).

Previously, MXC has been shown to elicit estrogenlike effects on the female reproductive system on both the gross and light microscopic level (5). It is now apparent that the effects of this pesticide are also exhibited at the subcellular level. Again, the observed effects closely mimic those of estrogenic substances. Even though MXC does exhibit estrogenic effects similar to DDT and chlordecone, the general toxicity of all of these agents may be quite different.

Whether the observed ultrastructural alterations in the organelles and the increased lipid accumulation observed in specific cellular compartments within the ovary following 4 week exposure to MXC affect the immediate fertility of the animal is not known. Whether such effects are reversible once exposure ceases is not known. What is clear, however, is that the ovary of an *adult* nonpregnant female is a target for the effects of MXC, which effects appear to be manifested by an interference with the process of steroid biosynthesis.

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