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CHLORDECONE-INDUCED FOLLICULAR TOXICITY IN MOUSE OVARIES

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Abstract—The effect of the pesticide, chlordecone, on murine follicular development was examined. Female CD-1 mice were exposed to chlordecone for 5 consecutive days for each of 4 consecutive weeks (0.25 mg/day). Controls received sesame oil vehicle or estradiol-17β (E-17β; 0.1 mg/day) since chlordecone has been ascribed estrogenic activity. Animals were sacrificed 24 h following the final exposure. Ovaries were removed, serially sectioned, and stained. Follicles were classified as small, medium, or large and were tabulated. Twice as many medium-sized follicles were found in the E-17β-treated mice as in both the chlordecone-exposed and sesame oil control groups. Both pesticide- and E-17β-exposed mice displayed a much higher percent of atresia in the large follicles; however, there were more actual healthy, large follicles in the E-17β group. Thus, both chlordecone and E-17β induced increased atresia among large follicles, which could be due to the estrogenicity of these agents. However, a decreased pool of healthy large- and medium-sized follicles occurred in chlordecone-treated mice, a condition not seen in E-17β-treated mice. Thus, the pool of potentially ovulatory follicles is reduced in the pesticide-treated animals.

Key Words: Chlordecone, Ovary, Follicle, Toxicology.

INTRODUCTION

Chlordecone (Kepone), a polycyclic chlorinated pesticide, is characterized as a reproductive toxin, since exposure to it has been shown to result in infertility in both male and female rodents (1,2). The cause of this infertility has eluded investigators. Some investigators reported this loss of fertility to be linked to the induction of persistent vaginal estrus (PVE) (1,3,4). A mouse is considered to be in PVE when its vaginal smear exhibits a cornified appearance for 4 consecutive days. Huber (1) theorized that it was a chlordecone-induced block of the preovulatory LH surge that caused the PVE. More recently, Uphouse and coauthors (5) observed that PVE occurred even in chlordecone-treated mice without blockage of the proestrus surge of LH.

Estrogenic activity has been attributed to chlordecone, since it has an affinity for binding to estrogen receptors in the cytosol (6) and since it increases uterine weight when administered to immature rodents (7). Swartz and coauthors (8) reported that mice exposed to either chlordecone or estradiol-17β (E-17β) both displayed PVE as early as 10 days of exposure; however, when these groups of animals were induced to ovulate with a superovulatory regimen of pregnant mare's serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) following a 4-week exposure to chlordecone, those mice exposed to chlordecone failed to respond normally, whereas E-17β treated animals ovulated large numbers of oocytes in response to the exogenous gonadotropins. This suggested that, although both chlordecone and estrogen were exhibiting similar effects on vaginal cytology, different effects might be exhibited on the ovary. Such effects appear to be due not to the estrogenic activity of the chlordecone but rather to its toxic effects.

The fact that fewer oocytes were stimulated to ovulate in chlordecone-exposed mice implies that the infertility observed in such animals in earlier studies resulted from fewer oocytes being ovulated. However, the reason for an absence or a reduced number of ovulated oocytes from chlordecone-exposed animals is not known. It could be due to a reduction in the pool of oocytes that respond to the gonadotropic stimulus, or there could be a pesticide-induced effect directly on the ovulatory process. Although the effects of many toxic agents on the female reproductive system have been evaluated, little attention has been directed toward examining the morphologic status of the components of the pesticide-exposed adult ovary. A few reports exist on the histologic condition of ovaries exposed to pesticides, but these exposures took place in the neonatal period. Heinrichs and coauthors (9) observed cystic follicles and a reduced number of corpora lutea in rats exposed neonatally to DDT. Gellert (7) reported an increased incidence of cystic follicles in ovaries exposed to DDT neonatally. The purpose of this study was to perform a detailed histologic assessment of the ovary following exposure of the adult mouse to chlordecone for 4 weeks in order to detect a morphologic basis for the decreased

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ovulatory response observed following exogenous gonadotropin stimulation.

MATERIALS AND METHODS

Adult virgin female CD-1 mice (Charles River Breeding Laboratories, Wilmington, MA) were used in this study. Mice, aged 7 to 10 weeks, were housed in animal quarters with a 14:10 light:dark cycle. Food and water were provided *ad libitum*. After a 7 day period of acclimatization, mice were randomly distributed into 3 major treatment groups: 1) a chlordecone-treated group, 2) a group treated with estradiol-17β, and 3) a sesame oil vehicle control group.

Mice were exposed to these agents for 4 weeks. Weekly procedures consisted of 5 consecutive daily exposures followed by 2 days of no treatment. This timetable was established to mimic an ordinary five-day work week, which would represent the maximum weekly exposure to which a female working with such a compound might be subjected.

Chlordecone (98% purity; Chem Service, West Chester, PA) was dissolved in sesame oil and administered in a 0.25 mg dose (8 mg/kg). This dosage has been shown to inhibit the ovulatory response to exogenous gonadotropins without eliciting any observable neurotoxic effects during the duration of exposure (8). Estradiol-17β (E-17β) (Sigma, St. Louis, MO) was administered at a dose of 0.1 mg dissolved is sesame oil. Control mice received the sesame oil vehicle only. All compounds were administered by oral gavage in a 0.2 mL volume of sesame oil.

Animals were sacrificed by cervical dislocation 24 h following the final exposure of the 4th week. Both ovaries were removed and were prepared for histologic evaluation. Ovaries were fixed in Bouin's fixative for approximately 72 hours. Following alcohol dehydration, tissues were embedded in paraffin, then serially sectioned at 8 μm and stained with hematoxylin and eosin. Sections of both ovaries of each mouse were examined under a light microscope, and the general histological appearance of the ovary was assessed. Follicles were classified according to Chen and coauthors' (10) modification of the method of Pederson and Peters (11). This classification is as follows:

Small — smallest oocyte still without follicle cells to an oocyte surrounded by no more than a single layer of follicle cells;
Medium — those containing growing oocytes surrounded by more than one layer of follicle cells and having no antrum;
Large — antral follicles including preovulatory ones.

These follicle counts were performed by examining every 10th section of each ovary and tabulating only those follicles in which the nucleus of the oocyte was visible. These data were then compared and statistical significance was determined using the Student *t* test.

Table 1. Mean numbers of follicles present in both ovaries of mice following 4-week exposure to chlordecone

Treatment	No. of mice	Types of follicles* Small	Medium	Large
Chlordecone	11	190.1 ± 32.8†‡§	103.8 ± 11.8§	27.5 ± 3.2
Estradiol-17β	7	368.0 ± 47.5	231.9 ± 41.0‡	28.0 ± 8.3
Sesame Oil	9	279.2 ± 39.6	116.2 ± 7.8	21.3 ± 2.5

*Tabulations made on every 10th section.
†Standard error of the mean (SEM).
‡$P < 0.05$ (chlordecone or estradiol-17β compared to sesame oil).
§$P < 0.05$ (chlordecone vs estradiol-17β).

While compiling the data, it appeared as if there were a large number of large follicles undergoing atresia in the ovaries of chlordecone-treated mice. In order to statistically verify this observation, the ovaries were reexamined. This time each section of both serially sectioned ovaries was examined for the presence of large follicles over 300 μm in their widest diameter. Follicles were classified as healthy or atretic according to the characteristics described by Mandl and Zuckerman (12,13) for antral follicles. Specifically, this characterization consisted of seeing more than three pyknotic cells in the granulosa cell layer, a free-floating oocyte detached from the granulosa cells, or an obviously degenerated oocyte. The percentage of atretic large follicles was determined and then compared among the three groups for statistical significance using the Student *t* test.

RESULTS

The data to be presented here consist of not only a comparison of the data between chlordecone-treated mice and those treated with the sesame oil vehicle control, but also between the pesticide-exposed mice and the E-17β-treated mice. This latter comparison is intended to determine whether any of the observed effects elicited by the chlordecone might be due to its inherent estrogenic activity.

In tabulating the number of different-sized follicles in the 3 different groups of animals, there were no differences in the total number of large follicles present when examining every 10th section (Table 1). With respect to medium-sized follicles, sesame oil controls contained a mean of 116.2 ± 7.8 follicles, which did not differ from the chlordecone-treated mice (103.8 ± 11.8). The number of medium-sized follicles in the E-17β group was significantly higher than both sesame oil controls and chlordecone-treated mice. In fact there were twice as many medium-sized follicles in the estrogen-treated animals as in each of the other two groups (Table 1).

Table 2. Mean number and condition of large follicles in both ovaries of mice exposed to chlordecone for 4 weeks

Treatment	Large follicles*			
	Total	Healthy	Atretic	% Atretic
Chlordecone	58.7 ± 5.8†	18.5 ± 1.9‡§	40.1 ± 5.1	68.3
Estradiol-17β	69.6 ± 6.7	25.4 ± 2.7	44.2 ± 4.3‡	63.5
Sesame Oil	58.1 ± 7.3	28.4 ± 6.0	29.7 ± 3.4	51.1

*Tabulations made on every section.
†Standard error of the mean (SEM).
‡$P < 0.05$ (chlordecone or estradiol-17β compared to sesame oil).
§$P < 0.05$ (chlordecone compared to estradiol-17β).

A significantly reduced number of small follicles was observed in chlordecone-treated mice when compared to both E-17β and sesame oil control mice (Table 1). The number of small follicles found in E-17β-treated mice was not significantly different from that in sesame oil controls.

When evaluating only large follicles in every section of the ovaries, it became readily apparent that there was a high percentage of large follicles that were atretic not only in chlordecone-treated mice (68.3%), but also in the E-17β-exposed mice (63.5%). Sesame oil control animals displayed a much lower percentage (51.1%) of visible atresia in their large follicles (Table 2). Since the actual numbers of large atretic follicles were quite similar in chlordecone and E-17β treated mice and since the total number of large follicles (healthy and atretic) present, when examining every section, was greater in ovaries from E-17β treated mice, it follows that the mean number of healthy large follicles in mice treated with E-17β (25.4 ± 2.7) was greater than that of chlordecone-treated mice (18.5 ± 1.9) (Table 2).

DISCUSSION

The ovary of the adult mouse exposed to 0.25 mg chlordecone for 4 weeks fails to respond normally to exogenous gonadotropins (8). This diminished response could have its basis in an alteration of a specific reproductive process or a combination of several such functional activities. Possible explanations include a decrease in the pool of oocyte/follicle cell complexes able to respond to the gonadotropins, possibly due to an alteration in endogenous gonadotropin secretion during the chlordecone exposure. Chlordecone could also induce a physical impediment to ovulation such as occurs in the luteinized unruptured follicle syndrome where granulosa cells undergo premature luteinization (14). This chlorinated pesticide could also alter receptor sites for gonadotropins on ovarian cells.

Chlordecone has been shown to possess estrogenic activity (6). Chlordecone produces disturbances in reproductive parameters similar to those induced by estradiol-17β. Estrogenic substances have been reported to disrupt the ovulatory responses in laboratory animals when such animals are exposed either prenatally or postnatally. Prenatal exposure of mice to diethylstilbestrol resulted in average numbers of follicles of all sizes; however, these females produced 70% fewer ova than controls when stimulated by gonadotropic hormones (15). Gellert (7) reported that neonatal exposure of rats to chlordecone induced precocious vaginal opening and anovulation. The induced anovulation caused by these agents when administered to prenatal or newborn animals is a result of the disruption of the normal differentiation of the hypothalamic-pituitary system, which in turn alters or inhibits normal secretion of both FSH and LH, gonadotropins necessary for normal follicular development and ovulation (16). However, very little information is available regarding exposure of adult mice to estrogenic compounds after the hypothalamic–pituitary axis is fully differentiated.

The data presented here show interesting differences when the numbers of the different-sized follicles are compared between chlordecone-exposed mice and controls. Two types of controls were employed in this experiment: the first, a vehicle control consisting of sesame oil only; the second, an E-17β control. The use of the latter group is imperative since chlordecone, like its predecessor, DDT, has been shown to induce effects similar to that of estrogen, such as persistent estrus, vaginal cornification, and uterine hypertrophy (7,17). Comparison of chlordecone- and E-17β-treated groups would serve to distinguish between effects resulting from the estrogenicity of the compound and those arising from its inherent toxicity.

Significant differences were observed in the populations of different-sized follicles. The significantly lower number of small follicles seen in the ovaries of chlordecone-treated mice could result from a high rate of induced atresia and subsequent disappearance of some of these small entities during the 4-week exposure period. This is similar to the toxic effects seen in mice exposed to a diet high in galactose where this sugar had a deleterious effect on small oocytes (10). Similarly, Mandl (18) and Krarup (19) found toxicity directed toward small oocytes by polycyclic aromatic hydrocarbons.

The number of medium-sized follicles in chlordecone-treated mice did not differ from controls. However, a mean of more than twice as many was found in the E-17β-treated group. This large number of middle-sized follicles could have been due to either a stimulation of the pool of small follicles to undergo hyperplasia and/or an inhibition of further development of some medium-sized follicles into the large-follicle pool. The tabulations of the total number of large antral follicles revealed a significantly smaller number of healthy follicles in the chlordecone-treated mice than in the E-17β group or the sesame oil controls. The percentages of large atretic

follicles in chlordecone- and E-17β-treated animals were similar. The fact that estrogen induces atresia in preovulatory follicles has been demonstrated by Clark and coauthors (20) and Krey and Everett (21). Ataya and coauthors (22) demonstrated somewhat similar findings in animals exposed to cyclophosphamide. They found the total number of large antral follicles to be lower in treated groups than in controls, and more atretic follicles were seen. However, in none of these studies was an attempt made to tabulate the number of healthly follicles present.

In the present study the number of healthy large antral follicles was greater in the E-17β-treated mice than in the pesticide-exposed animals. This coupled with the larger number of medium-sized follicles in estrogen-treated mice could account for the normally observed induced ovulatory response, thereby offsetting the high number of atretic large follicles found in the estradiol-treated ovaries.

Thus, both chlordecone and E-17β cause an increase in atresia in the large follicles, which is probably due to the estrogenicity of these agents. However, the decreased number of healthy large follicles and medium-sized follicles in chlordecone-treated mice appears to be due to the toxicity of this agent. As a result, the pool of oocytes available for ovulation is reduced, and response to exogenous gonadotropins is reduced. Whether this response is reversible once chlordecone exposure ceases is not known from these studies.

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