Destruction of primordial ovarian follicles in adult cynomolgus macaques after exposure to 4-vinylcyclohexene diepoxide: a nonhuman primate model of the menopausal transition

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Objective: To determine whether the 4-vinylcyclohexene diepoxide (VCD)-treated mouse menopause model, which involves accelerated atresia of primordial follicles and induces gradual ovarian failure (while sparing the ovarian stroma), can be adapted to nonhuman primates.
Design: Controlled periclinical trial (nonhuman primates).
Setting: Comparative Medicine Clinical Research Center.
Animal(s): Four adult female cynomolgus monkeys.
Intervention(s): Once-daily IM injections for 15 days as follows: vehicle or VCD doses of 80 mg/kg, 160 mg/kg, 250 mg/kg. Ovaries were removed 27 days after treatment, and pathological determinations were made at necropsy.
Main Outcome Measure(s): Baseline and interim hematologic and biochemical measures, physical exams, and body weights. Follicle counts and organ evaluation at necropsy.
Result(s): A nearly complete elimination of primordial, intermediate, primary and secondary follicles was achieved with 250 mg/kg VCD. A 50% reduction in primordial and primary follicles was observed with 160 mg/kg VCD. No effect of 80 mg/kg VCD per day was observed. Clinical health measures remained within normal range except for transient, mild increases in liver enzymes and an inflammatory response at the injection site with 250 mg/kg. Postmortem evaluations (9 months) revealed no gross or histological lesions in the organs studied.
Conclusion(s): These results demonstrate that the monkey ovary is susceptible to VCD and that as in rodents, primordial and primary follicles are targeted selectively. (Fertil Steril® 2006;86(Suppl 3):1210–6. ©2006 by American Society for Reproductive Medicine.)

Key Words: Primordial follicles, ovaries, 4-vinylcyclohexene diepoxide, monkeys, menopause

It has been estimated that the world's population of postmenopausal women (most of whom reach this state naturally rather than surgically) will more than double between 1990 and 2030 (1). Given the current average lifespan, these women could spend several decades in a hypoestrogenic state, as a result of which they are likely to be affected increasingly by the progression of osteoporosis, cardiovascular disease, and cognitive deficits. Intervention with either estrogens or alternative treatments before, during, or just after the menopausal transition may slow the development of these chronic diseases and related pathobiologic processes (2). Unfortunately, the most frequently used approach for modeling menopause, animal ovariectomy, does not produce a condition that mimics the natural progression through perimenopause into menopause, the gradual process by which the vast majority of women reach menopause between the ages of 40 and 55 years.

In this study, we addressed the foregoing deficiencies in menopausal models by extending to cynomolgus monkeys (*Macaca fascicularis*) a technique developed in mice and rats involving exposure to the occupational chemical 4-vinylcyclohexene diepoxide (VCD). This chemical compound selectively destroys ovarian primordial and primary follicles by accelerating the natural process of atresia (3–6). Because follicle loss is selective, the mouse undergoes gradual ovarian failure, and ultimately this procedure results in a follicle-depleted animal that retains residual ovarian stromal tissue with the potential to produce steroid hormones (7). Although the VCD-treated mouse represents a significant research advance, this model has characteristics that limit its applicability for research on some issues in women's health. Unlike anthropoid primates, which have a menstrual cycle of

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approximately 28 days that is characterized by cyclic sloughing of endometrial cells, the mouse estrus cycle occurs every 4–5 days, and sloughing of uterine epithelial cells does not occur.

These and other substantial physiological and anatomical differences between mice and primates limit the usefulness of the mouse for modeling human-like changes in lipids and lipoproteins, the development of coronary artery atherosclerosis, breast and uterine proliferation, and sociosexual and cognitive phenomena. Recognizing these limitations prompted us to explore whether VCD's effects on the nonhuman primate ovary would be like those reported in mice, thus allowing the development of a monkey model of perimenopause and postmenopause that would closely resemble midlife hormonal events as they occur in women.

MATERIALS AND METHODS

Animals

Four female cynomolgus monkeys (*M. fascicularis)* of similar age (8–12 years) were used for this study. Epiphyseal closure (and thus full skeletal maturity) occurs at approximately 9 years of age in this species, whereas the reported maximum achievable life span of cynomolgus monkeys is >30 years (8). The monkeys were imported directly from the Indonesian Primate Center (Pusat Studi Satwa Primata) at the Institut Pertanian Bogor in West Java, Indonesia.

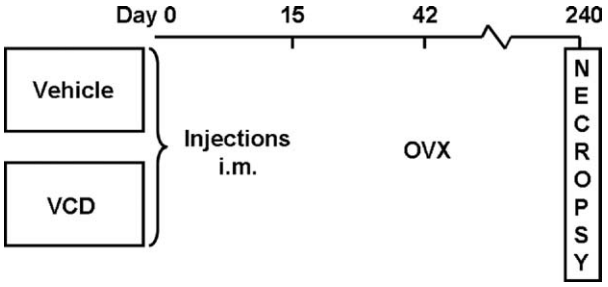
The monkeys were fed an isoflavone-free, purified diet prepared in our laboratory that contained 20% of calories as protein (equal parts casein and lactalbumin), 30% of calories as fat (mixture of saturated and unsaturated fats), and 50% of calories as carbohydrates (dextrin, sucrose, and wheat flour). Vitamin Mix AIN-93-VX and Mineral Mix AIN-93-MX also were added to the diet (Harlan Teklad, Madison, WI) (9). The monkeys were fed 120 kcal of diet per kilogram of body weight. All animal procedures were performed in accordance with federal, state, and institutional guidelines, and this study was approved by the Institutional Animal Care and Use Committee of Wake Forest University.

Study Design

The design of the study is shown schematically in Figure 1. The four fully mature female monkeys used for this study were of similar age (8, 9, 12, and 13 years). Archived ovaries from two premenopausal monkeys (8 and 13 y old) were used as additional untreated comparators. The monkeys received IM injections for 15 consecutive days as follows: vehicle (sesame oil; n = 1) and VCD doses of 80 mg/kg (n = 1), 160 mg/kg (n = 1), or 250 mg/kg (n = 1). The ovaries were removed for follicle counting 27 days after the last VCD injection (day 42). Measures of health profile data (hematologic, biochemical, physical exam, and body weight) were collected at baseline and on days 4, 7, 12, 15, 21, 28, 35, 75, and 146. After 240 days, the monkeys were killed, and a complete diagnostic necropsy was performed. No hormonal data were collected during the 27-day interval between VCD treatment and ovariectomy, because no changes other than cyclic variation would be expected in this short period of time.

FIGURE 1

Study design. Three monkeys were given IM injections of VCD daily for 15 days as follows: 80 mg/kg (n = 1), 160 mg/kg (n = 1), and 250 mg/kg (n = 1). One monkey was injected daily with vehicle only (sesame oil). Twenty-seven days after the last injection, all monkeys had their ovaries removed (OVX), and approximately 9 months later, they were necropsied.



Appt. Ovarian follicle destruction in monkeys. Fertil Steril 2006.

Health Profile Data

Hematologic and biochemical measures were performed at the Comparative Medicine Clinical Research Center (CMCRC) laboratory using Hemavet 950FS (Drew Scientific) and Ace-Alera (Alfa Wassweman, Diagnostic Technologies, LLC) analyzers. Hematologic measures included the following: total white blood cell count, platelets, hemoglobin, hematocrit, red blood cell count, mean red cell volume, segmented neutrophils, band cells, lymphocytes, monocytes, eosinophils, and basophils. Biochemical measures included the following: blood urea nitrogen, glucose, total serum protein, albumin, total bilirubin, creatinine, alkaline phosphatase, aspartate amino serum transferase (AST), amino alanine transferase (ALT), and electrolytes (Ca, P, Na, K). In addition, physical examinations were performed at the time of blood collection. The physical examinations included the following: auscultation of heart and lungs, assessment of hydration and mucous membrane color, visual inspection of integument, abdominal palpation, body temperature, and body weight. Animals were monitored daily for signs of central nervous system changes (lethargy, drowsiness) and motor defects (i.e., gait abnormalities, muscle weakness).

Ovariectomy and Follicle Counts

Ovaries were removed by laparotomy for histological evaluation. The monkeys were sedated with ketamine HCl (15 mg/kg), given ketoprofen for analgesia (5 mg/kg IM) before