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An Acute Oral Gavage Study of 3β-Acetoxyandrost-5-ene-7,17-dione (7-oxo-DHEA-acetate) in Rats

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The present study was done to assess the tolerance of rats for 3-acetoxyandrost-5-ene-7,17-dione (7-oxo-DHEA-acetate, 7-ODA) when administered as a single oral gavage dose. Five groups of Sprague-Dawley rats (Crl:CD (SD) BR VAF/Plus) (five/sex/group) were treated with 7-ODA at a dose level of 0 (control), 250, 500, 1000, or 2,000 mg/kg of body weight in a dose volume of 10 ml/kg. Food and water were provided ad libitum. All animals survived in good health to the scheduled sacrifice on Day 15. The single oral administration of 7-ODA had no apparent effects on body weight. Food consumption was significantly higher for all female treated groups during week two; however, the statistically significant differences were not considered to be of clinical consequence. Treatment caused no apparent changes of gross or microscopic anatomical structures of nine different organs. This study demonstrated that the no-observable adverse effect level for a single oral dose of 7-ODA in male and female rats was 2,000 mg/kg.

Dehydroepiandrosterone (DHEA) is synthesized in the primate adrenal cortex from cholesterol via pregnenolone by the action of the adrenal cytochrome P450-17α. It is secreted and circulates almost entirely as the sulfate ester and is the most abundant steroid in human blood (2). Adult rodents lack 17-hydroxylase activity in their adrenals, their blood contains little or no DHEA, but they respond to administered DHEA. In some systems the free steroid is thought to be the cellularly-active form (3,4), but both DHEA and its sulfate ester have distinctive effects on some systems, e.g., neurite growth (5).

Several positive effects have been recorded in studies utilizing a wide range of doses of DHEA and DHEAS including weight loss in obese animals (6,7), memory-enhancing effects in mice (8,9), tumor prevention and growth inhibition (10,11), enhancement of bone density in post-menopausal women (12) and up-regulation of the immune system (3,4,13,14). These diverse beneficial effects of DHEA deserve further development.

DHEA and DHEAS have been used in clinical trials to treat a number of conditions including cancer (15), multiple sclerosis (16), hyperlipidemia and coronary disease (17,18), lupus (19), Alzheimer's disease (20), and HIV/AIDS (21,22). In some subjects it may be useful in treating menopausal and PMS symptoms and as a mood and energy-booster (16,23,24).

Several adverse effects of DHEA have been seen in humans and experimental animals including elevated plasma androgen concentration and the resulting hirsutism in women (16,23,24), increased tumor incidence (25–27), and liver histological changes (28–32). Inconsistently-reported side effects of DHEA in humans include headaches, insomnia, nausea, fatigue, rash, diarrhea, increased nasal congestion and vomiting. Elevated plasma sex hormone concentrations, uterine enlargement and polycystic ovaries are seen in rats given DHEA (33,34).

The deleterious effects of DHEA and the large doses required to obtain responses limit its possible clinical use and encourage the search for more therapeutically efficacious steroids. Both chemically prepared (35,36) and naturally occurring (37,38) derivatives of DHEA have been investigated. The most active of these is 7-oxo-DHEA (36,39). This steroid was first found in human urine by Lieberman et al. (40); it is produced in normal subjects and in larger amounts by persons with adrenal abnormalities (1,41). It is secreted by the adrenal as the sulfate ester (42).

The 3-acetyl ester of 7-oxo-DHEA was used as the test material for this study because esterification is

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Abbreviations used: 7-ODA or 7-oxo-DHEA-acetate, (3β-acetoxyandrost-5-ene-7,17-dione); DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone-3-sulfate; MC, methyl cellulose; Tween 80, polyoxyethylene sorbitan monooleate. (The abbreviation 7-OD for 7-oxo-DHEA was first used by Gallagher (1)).

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required during the chemical preparation of 7-ODA and deesterification after introducing the 7-oxo group serves no useful purpose. Acetyl and other esters of these steroids are readily hydrolyzed by esterases in blood and tissues with the result that most esters are as active as the free steroid (36).

METHODS AND MATERIALS

Methods. This study was designed in accordance with the United States Food and Drug Administration's Good Laboratory Practice Regulations for Nonclinical Laboratory Studies, 21 CFR 58, with the exception of dose analysis, quality assurance involvement, and test material characterization. The studies were conducted at Covance Laboratories Inc., Madison, Wisconsin (protocol TP5299).

Materials. The test material, 3β-acetoxyandrost-5-ene-7,17-dione (7-ODA), a white crystalline powder, was synthesized by one of the authors at the University of Wisconsin as described (36). Its purity was determined to be 99.8% by HPLC (Albany Molecular Research, Albany, N.Y.).

The carrier was a mixture of 1% methylcellulose (MC) and 0.1% Tween 80 (polyoxyethylene sorbitan monooleate) (Sigma Chemical Co., St. Louis, MO) in reverse osmosis water. The carrier mixture was prepared once using weighed amounts of Tween 80 and MC added to a portion of RO water. A clear, homogeneous mixture was obtained using a Brinkman polytron homogenizer (Model PT 10/35; probe PTA 10TS). After dilution to the required volume, a uniform mixture was maintained by magnetic stirring. The carrier mixture was stored at 5°C.

The 7-ODA solutions in various concentrations were prepared the day of administration. The steroid was pulverized with a morter and pestle and the requisite amount was transferred to a beaker containing a portion of the carrier. This suspension was mixed for at least 3 minutes in the polytron homogenizer, then degassed in a vacuum chamber until foaming ceased. Additional carrier was added to provide a suspension of 200 mg 7-ODA/ml. This solution was used for group 5; for groups 2 through 4 the required volume of the stock solution in a graduated cylinder was diluted to provide the respective doses in a volume of 10 ml/kg body weight.

Animals. Twenty-nine male and 29 female Sprague-Dawley (Crl:CD (SD) BR VAF/Plus) healthy rats from Charles River Laboratories, Inc. (Portage, Michigan) were acclimated for 7 days before initiating treatment. During this time the animals were examined for any health abnormalities. The rats were approximately 5 weeks old at initiation of treatment (day 0); males weighed 136–137 g and females weighed 111–129 g. They were housed individually in stainless steel, screen-bottom cages in a room maintained at 19 to 25°C with a relative humidity of 50% ± 20% and a 12 hour light/dark cycle. Each rat was implanted with an individually-coded passive integrated transponder and numbered for identification.

Certified Rodent Diet #5002 (PMI Feeds, Inc., Richmond Indiana) was provided ad libitum unless otherwise specified. The water provided ad libitum was analyzed for heavy metals, organophosphates, chlorinated hydrocarbons, total dissolved solids and specified microbiological content. No contaminants that would have interferred with this study were found in either food or water.

Treatment. On Day 3, after a veterinarian examination, the rats were weighed and ranked by body weight. The 25 of each sex whose weight deviated least from their mean were retained for the study. The rats were allocated to groups based on random numbers. Group mean body weights were analyzed using Bartlett's test for homogeniety of variance at the 5.0% probability level (43) and found to be homogeneous. All data for an animal were recorded under its number.

Five groups of rats, 5 males and 5 females in each, received doses of 0, 250, 500, 1000 and 2000 mg/kg respectfully. These were administered in solutions of 0, 25, 50, 100, and 200 mg/ml at a dose volume of 10 ml/kg. The gavage route of administration was used because the intended route of administration to humans is oral. Dose preparations were given on Day 1. The steroid mixtures were maintained during these procedures by magnetic stirring.

Observation of animals. Each rat was examined twice daily (a.m. and p.m.) for possible signs of poor health or abnormal behavior. At least once weekly each animal was removed from its cage and examined; any unusual or abnormal findings were recorded. Individual body weights were recorded on the day of treatment and weekly thereafter. Food consumption was recorded weekly.

Anatomical pathology. On day 15, after overnight fasting, the rats were weighed, anesthetized with pentobarbital, exsanguinated, and necropsied in random order. The necropsy included a macroscopic examination of the external surface of the body, all orifices, the cranial cavity, the external surfaces of the brain and spinal cord, the nasal cavity and paranasal sinuses, the viscera, and the thorasic, abdominal and pelvic cavities. The adrenals, brain, heart, kidneys, liver, spleen, ovaries, testes, and thymus were weighed. Paired organs were weighed separately. Organ to body weight percentages and organ to brain weight ratios were calculated. The adrenals, brain, heart, kidneys, liver, spleen, and thymus of each animal were preserved in phosphate-buffered 10% formalin, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically.

Statistical analysis. All data collected on or after the first day of treatment were analyzed. Levene's test (44) was used to test for variance homogeneity. In the case of heterogeneity of variance at $p \leq 0.05$, transformations were used to stabilize the variance. Analysis of variance (43) was done on the homogeneous or transformed data. If the ANOVA was significant, Dunnett's multiple comparison t-test (45) was used for pairwise comparisons between treated and control groups. One-way ANOVA was used to analyze body weights, cumulative body weight gains, food consumption, organ weights, organ-to-body weight percentages, and organ to brain weight ratios. Group comparisons were evaluated at the 5.0% two-tailed probability level.

RESULTS

Survival and antemortem observations. All of the animals survived to the scheduled sacrifice on Day 15. No abnormal behavior, altered appearance or abnormal symptoms were observed during the weekly examinations. There were no differences in food consumption attributable to 7-ODA in the male groups. Statistically significant higher food consumption occurred in all treated female groups during week 2 (Table 1); however the differences were not considered to be toxicologically important. There were no differences in body weights or cumulative weight gains attributed to 7-ODA (Table 1).

Anatomical pathology. There were no significant differences between groups in the weights of their nine different organs that were examined. As a result of minor deviations of both body weight and right testis weight, the right testis weight-to-body weight percentages were somewhat lower in males given 250 or 1,000 mg/kg doses. In the absence of significant weight differences in the contralateral organ and the absence of correlating macroscopic or microscopic changes, these weight ratios were considered incidental and unrelated to the test material. Macroscopically and mi-

TABLE 1

Mean Body Weights and Food Consumption

	Male					Female				
Sex Group	1	2	3	4	5	1	2	3	4	5
7-ODA, mg/kg	0	250	500	1000	2000	0	250	500	1000	2000
Wt. gm, day 0	149	150	152	149	148	122	121	119	125	120
Wt. gm, day 15	286	286	289	283	277	182	180	196	196	183
Food, gm; week 2	208	207	208	207	200	12	141*	146*	144*	141*
Standard deviation						(1.9)	(9.7)	(12.4)	(4.4)	(3.2)

Note. Rats that had spilled food were excluded from the average consumption data.
* Group mean is significantly different from the mean of the control group (group 1) at $p \leq 0.05$.

croscopically there were no abnormal changes in the brains, adrenals, hearts, and thymi of any of the animals. One female in each of groups 2 and 4 had fluid in its uterus; one male in each of groups 2 and 4 had an enlarged kidney pelvis, and one control female had an opaque capsule on its spleen. Microscopic examination disclosed some kidney tubular mineralization in two control females, in four group 2 females, and in three females in each of groups 4 and 5. Several of the animals in the control and all treated groups, males and females, exhibited mild liver lymphoplasmatic infiltration or lymphohistiocytic inflammation. One female in the control group had capsular fibrosis in its spleen. There were no major differences between control and treated animals and no correlation with dose level in the incidence of any finding. A single oral administration of 7-ODA to rats had no apparent toxic effect on either macroscopic or microscopic anatomy.

DISCUSSION

This study showed that the steroid 7-oxo-DHEA-3-acetate (7-ODA), at single oral dose levels up to 2,000 mg/kg, had no observable, serious, adverse effects on either male or female rats. Liver-to-body weight and liver-to-brain weight ratios were not altered by treatments with 7-ODA. Microscopic examination of the several internal organs also disclosed no alterations that could be assigned to the steroid treatment.

There have been no comparable, single-dose toxicity studies done with DHEA; adverse effects resulting from prolonged treatment with DHEA have been described in the introduction to this paper. The results obtained lead us to conclude that the no-observable-adverse-effect for a single oral dose of 7-ODA in male and female Sprague-Dawley rats is 2,000 mg/kg. Studies (manuscript in preparation) using this compound in a double blind, placebo-controlled human clinical trial have disclosed no adverse responses to doses up to 200 mg daily for four weeks.

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