Biochemical and Biophysical Research Communications **254,** 124–126 (1999)
Article ID bbrc.1998.9908, available online at http://www.idealibrary.com on IDEAL®

An Escalating Dose Oral Gavage Study of 3β-Acetoxyandrost-5-ene-7,17-dione (7-oxo-DHEA-acetate) in Rhesus Monkeys

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Received December 8, 1998

To test the effects of 7-oxo-dehydroepiandrosterone-3 acetate (hereafter 7-ODA) in Rhesus macaques the steroid was administered by oral gavage to two male and two female monkeys. Dose levels of 250, 500, and 1,000 mg/kg body weight (BW)/day were administered on days 1, 3, and 5 respectively, and 1,000 mg/kg on days 7 through 11. Each group received the dose in a volume of 10 ml/kg BW. All animals survived to the scheduled sacrifice on day 12. No adverse clinical effects of 7-ODA were observed at the 250 or 500 mg/kg doses. Females vomited on non-treatment days and all animals vomited on some days after being given the 1000 mg/kg dose. Excessive salivation was observed before or immediately after dosing on days 9 through 11. Appearance, behavior and body weights were not altered by the treatments. Visual examination of all body cavities, and macroscopic and microscopic examination of 42 different organs and tissues found no lesions or abnormalities.

The diverse metabolic effects of DHEA have been briefly described in the accompanying paper (1). The many positive, potentially useful properties of this steroid are partially negated by the hazards it could introduce to some individuals as a result of it's being converted to either male or female sex hormones in greater than normal amounts. The most consistently-reported adverse effects seen in humans have been hirsutism and acne (2–4). A more suitable steroid for therapeutic purposes is 7-oxo-DHEA for it is more potent than DHEA in many of the desired functions, is not recognized by the androgen receptor in prostatic tissue (5), and cannot be converted to either testosterone (6) or estrogens (7). To prepare for possible use of this naturally-occurring steroid in humans we tested the tolerance of a primate species for 7-ODA. We have previously established that this acetyl ester is readily hydrolyzed in animals and is as effective as equimolar amounts of the free steroid (8). In the present study we find that 7-ODA in doses up to 500 mg/kg body weight were well tolerated and caused no significant changes in organ structure or blood composition. Such doses far exceed those that might be given to humans.

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, 21CFR 58, with the exception of the dose analysis. The study was conducted at Covance Laboratories Inc., Madison, Wisconsin (protocol TP 5301).

Materials. All materials and preparative procedures were as described in the accompanying paper (1).

Animals. Three male and three female rhesus macaques (HRP, Inc., Alice, Texas) were acclimated for at least 30 days before initiation of treatment. During acclimation the animals were examined for abnormalities, given three tuberculosis tests, a physical examination, and a fecal flotation test for parasites. They were housed at a temperature of 19° to 26°C, a relative humidity level of 50% ± 20%, and a 12-hour light/12 hour dark cycle. The animals were maintained individually in stainless steel cages. Their weights varied from 3.3 to 4.1 kg at initiation of treatment. Each animal was assigned a permanent number upon arrival and identified with an ear tag. All data for each animal were recorded under this number.

The monkeys were fed Certified Primate Diet #5048 (PMI Feeds, Inc., Richmond, Indiana) ad libitum. The diet was supplemented with bananas, apples and cereal (not quantified). Water was provided ad libitum; its quality was described (1).

Treatment. Two male and two female macaques received the test material 7-ODA by oral gavage at dose levels of 250, 500, and 1,000 mg/kg BW on days 1, 3 and 5 respectively. Dose concentrations were prepared as described in the accompanying paper. The oral gavage route was chosen because the intended route of administration to humans is orally. Because no clear evidence of toxicity was observed after a single dose of 1,000 mg/ kg, the animals were treated with 1,000 mg/kg for 5 consecutive days (days 7 through 11). All dose

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Abbreviations used: DHEA, dehydroepiandrosterone; 7-ODA or 7-oxo-DHEA, 3β-acetoxyandrost-5-ene-7,17-dione; MC, methylcellulose; Tween 80, polyoxyethylene sorbitan monooleate.

0006-291X/99 $30.00

TABLE 1

Blood Constituents Measured

Hematology	
Red blood cell count	Differential blood cell count
Hemoglobin	Reticulocyte count
Hematocrit	Corrected white cell count
Mean corpuscular volume	Segmented neutrophil count
Mean corpuscular hemoglobin	Band neutrophil count
Platelet count	Lymphocyte count
Prothrombin time	Monocyte count
Blood cell morphology	Eosinophil count
White blood cell count	Basophil count

Clinical Chemistry	
Calcium	Triglycerides
Chloride	Total protein
Inorganic phosphorus	Albumin
Potassium	Globulin
Sodium	Alkaline phosphatase
Cholesterol	Alanine aminotransferase
Glucose	Aspartate aminotransferase
Creatinine	Creatine kinase
Urea nitrogen	
Total bilirubin	

preparations were given in a volume of 10 ml/kg. The intubation tubes were flushed with 5 ml of water to ensure complete transfer. Individual doses were calculated based on daily recorded body weights. The test material mixtures were maintained during dose administration using a magnetic stir plate and stir bar.

Observation of animals. Each animal was observed twice daily (a.m. and p.m.) for signs of poor health or abnormal behavior. They were similarly observed at approximately 1 hour after each dose had been given. Effects were recorded as they were observed. Individual body weights were recorded weekly before treatment, on each dosing day, and on the day of sacrifice. Food consumption was confirmed qualitatively via daily inspection beginning at least one week before initiating treatment.

Clinical and anatomical pathology. After fasting overnight (water was provided ad libitum), each animal was bled from the femoral vein on day -7 (before initiating treatment), pre-dose on day 7, and on day 12 (at sacrifice). Table 1 gives a list of blood constituents measured.

On day 12, after overnight fasting, the animals were weighed, anesthetized with pentobarbital, exsanguinated, and necropsied in random order. The necropsy included a macroscopic examination of the external body surface, all orifices, cranial cavity, brain, spinal cord, nasal cavity and paranasal sinuses, viscera, and the thoracic, abdominal, and pelvic cavities. Tissues were preserved in phosphate-buffered formalin, imbedded in paraffin, sectioned and stained with hematoxylin and eosin, and examined microscopically.

RESULTS

Survival and antemortem observations. All four animals survived to the sceduled sacrifice on day 12. No adverse changes related to the test material were observed when 7-ODA was administered at 250 or 500 mg/kg; however, at 1,000 mg/kg excessive salivation was observed on day 9 in all animals before dosing, on day 10 in one male and both females immediately after dosing, and on day 11 in one male and both females before dosing. Both females vomited on days 2 and 4, and at 1,000 mg/kg each of the animals vomited at varying times. There were no testmaterial-related effects on body weight. Food consumption for all animals was normal with the exception of decreased consumption by both females on day 4 and for three out of four animals during the repeated dose phase at 1,000 mg/kg daily.

Anatomical pathology. No abnormalities were noted except that both females had a diffuse alopecia on the hind limbs. One female was in estrus. Forty two different organs and tissues were examined; all appeared to be normal.

Clinical pathology. At day -7 the concentration and/or appearance of blood constituents were within the normal range for young adult rhesus monkeys. After treatment was initiated there were several relatively small differences between pretreatment values and those on day 12 (post treatment). These differences were generally consistent for all animals studied and included lower red cell count, hemoglobin, and hematocrit and an increase in reticulocytes and platelets. The final concentrations were still in the normal ranges. No other hematological changes occurred.

Blood glucose increased from an average value of 70 mg/DL on day -7 to 100 on day 12; cholesterol decreased from 165 mg/DL to 124 and alkaline phosphatase decreased from 430 IU/L to 189 during the same time period. One female showed a striking increase in blood plasma creatine kinase. There were no significant alterations in the other blood constituents (Table I).

DISCUSSION

This study demonstrated that 7-oxo-DHEA-acetate (7-ODA) at dose levels of 250 and 500 mg/kg had no apparent adverse effects on rhesus monkey appearance, behavior, or body weight. The occurrence of lowered food intake at dose levels of 500 and 1,000 mg/kg did not result in body weight changes. Inconsistent effects of DHEA on body weight in animals and humans have been reported (9-15). In unpublished experiments (J. Kemnitz, H. Lardy et al.) feeding 140 mg of 7ODA/kg body weight daily to adult Rhesus monkeys for four weeks did not influence body weight or liver histology. Even that dose, smaller than used in the present study, is far greater than any amount likely to be used in humans.

Diffuse alopecia, observed at necropsy in both female monkeys, has also been seen in human females given DHEA (3).

The several relatively small differences in certain pre- and posttreatment blood values are of uncertain relationship to treatment. Confirmation of a definitive relation-

ship of these findings to 7- ODA was precluded by the absence of a control group which was omitted because of fund limitations. It is possible that some of the differences reflected normal day-to-day variability. None of these changes represented obvious health abnormalities or organ dysfunction, and values for individual animals were not atypical for young adult rhesus monkeys.

The occurrence of vomiting by both females on days 2 and 4 (when no active compound was administered) may indicate that vomiting at the higher dose was not caused by the steroid but by some other factor. The combination of steroid with the Tween detergent may have caused the reaction. 7-ODA taken for four weeks in doses of 100 mg bid by volunteers in a clinical trial did not cause nausia.

The increased liver mass found in rodents fed DHEA (16-19) was not manifest in our monkeys.

ACKNOWLEDGMENT

This investigation was supported by Humanetics Corp., Minneapolis, MN.

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