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SHORT REPORT

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Determination of dexamethasone dose for cortisol suppression in adult common marmosets (*Callithrix jacchus*)

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Abstract

We conducted a dose–response study of dexamethasone to investigate an optimal dexamethasone suppression test for common marmosets. Twelve marmosets received 0.1, 0.5, or 1.0 mg/kg dexamethasone. Doses of 0.5 and 1.0 mg/kg both suppressed endogenous cortisol for at least 18 h with greater individual variability in the lower 0.5 mg/kg dose.

KEYWORDS
common marmoset, dexamethasone suppression test, glucocorticoid

1 | INTRODUCTION

Dexamethasone (DEX), a synthetic glucocorticoid that preferentially binds *NR3C1*, suppresses endogenous adrenocorticotropic hormone (ACTH) and cortisol production. Dexamethasone suppression tests (DST) are used to evaluate the functioning of the hypothalamic–pituitary–adrenal (HPA) axis by assessing the integrity of the glucocorticoid feedback receptor sensitivity[1] and are utilized in basic research involving non-human primate models of stress and neuroendocrine dysfunction.[2–9]

Common marmosets (*Callithrix jacchus*) are increasingly used in biomedical research as valuable models of human disease, aging, and social behavior.[10,11] Studies utilizing DST in marmosets have largely investigated social suppression of cortisol in subordinate female marmosets, psychogenic stress, or consequences of prenatal DEX exposure[12-16], with male marmosets rarely being tested.[17,18] The DEX dose given to marmosets in previous studies has ranged broadly from 0.1 to 5.0 mg/kg. Johnson et al.[14] conducted a dose–response (0.1–4.0 mg/kg) study of DEX in male marmosets and female marmosets but only reported outcomes using 1.0 mg/kg. Interestingly, male marmosets and female marmosets were found to have differential responses to the DST test in certain social conditions.

Given the inconsistencies in the literature, we conducted a dose–response study of DEX to evaluate an optimal dose for use in marmoset DST and to examine, in an exploratory fashion, sex-specific responses of marmosets to DST.

2 | MATERIALS AND METHODS

2.1 | Animals and sampling

Twelve adult marmosets (six male, six female; mean age = 2.4 ± 0.8 years; Table 1) were selected from the Barshop Institute for Longevity and Aging Studies at UT Health San Antonio (UTHSA) colony. Animals were in stable, male–female nulliparous pairs, and deemed healthy by the veterinarian according to clinical and physical evaluation prior to the study. The Institutional Animal Care and Use Committee of UTHSA reviewed and approved the study, and the U.S. National Research Council's guidelines for the Care and Use of Laboratory Animals were followed.

On Day 1, female marmosets were injected intramuscularly (IM) with 0.75 µg estrumate (cloprostenol sodium; 0.15 ml of 1:50 dilution in sterile saline) as a means of standardizing ovarian cycle phase, with female marmosets expected to be in the luteal phase at the onset of DST. Apart from peri-ovulation, circulating cortisol levels in female marmosets are comparable between follicular and luteal phases of the ovarian cycle.[19] A baseline 1 ml blood sample was obtained from the femoral vein at 7 AM on Day 15. We used block randomization (block = sex) to assign marmosets to receive dexamethasone sodium sulfate (Henry Schein) dose of 0.1, 0.5, or 1.0 mg/kg; IM injections were administered at 1 pm on Day 15. A second blood sample was obtained on Day 16 at 7 AM. Animals were fasted overnight prior to each sample, and samples were placed into SST tubes. Plasma was separated from whole blood by centrifuging at 1000 *g* for 10 min and stored at −80° C. Progesterone (for female marmosets) and cortisol measurements occurred at the University of Wisconsin Assay Services Lab, as previously described.[6]

2.2 | Data analysis

The experimental design suggested a three-way analysis of variance, with sex and dose as between-subjects variables and pre-post as a within-subjects variable. Exploratory analyses revealed no statistically reliable effect of sex; therefore, we performed a two-way analysis of variance, with dose as between-subjects variable and pre-post as within-subjects variable. Cortisol concentrations were log-transformed due to heteroscedasticity of the data. Alpha was set at 0.05.

3 | RESULTS

Two-way ANOVA indicated circulating cortisol concentrations post-DEX treatment were significantly lower compared with pre-treatment values (pre-post: F (1, 9)=28.24, $p < .001$). A significant effect of dose was found (F (2, 9)=6.76, $p = .016$), with the 1.0 mg/kg dose resulting in significantly lower cortisol compared with the

TABLE 1 Cortisol concentration pre- and post-dexamethasone dosing for female and male common marmosets

Dexamethasone dose	Sex	Age (years)	Body weight (g)	Pre-Dose Cortisol (µg/dl)	Post-Dose Cortisol (µg/dl)	Progesterone (ng/ml)
0.1 mg/kg	F	2.8	340	78.5	87.5	23.75
	F	2.1	475	123.0	76.0	0.732
	M	1.6	456	36.7	42.2	N/A
	M	3.4	402	46.3	55.0	N/A
0.5 mg/kg	F	2.1	540	95.2	66.5	4.992
	F	3.4	372	105.6	49.8	0.928
	M	2.1	403	50.0	33.5	N/A
	M	2.6	374	150.0	79.0	N/A
1.0 mg/kg	F	3.4	427	61.5	8.4	29.00
	F	1.4	570	39.5	15.6	0.17
	M	1.3	464	94.2	3.0	N/A
	M	2.8	403	66.2	2.3	N/A

Note: Progesterone concentration is reported for female marmosets. Statistical analyses were performed on $\log_{10}$-transformed data.

0.5 mg/kg dose. There was a significant dose x pre-post-treatment interaction (F (2, 9)=9.17, p = .007). With the 0.1 mg/kg dose, there was no significant difference between pre-treatment and post-treatment cortisol levels (p = .819). At the next-higher (0.5 mg/kg) dose, post-treatment cortisol levels were lower than the pre-treatment levels (p = .024). With the highest dose, 1.0 mg/kg, post-treatment cortisol levels were lower than the pre-DEX treatment levels ($p < .001$). Table 1 presents the data for each animal in each treatment condition; raw data indicate that the highest dose (1.0 mg/kg) resulted in less variability in post-treatment cortisol concentrations (range: 2.3 to 15.6 ug/dl) than was the case for the 0.5 mg/kg dose (33.5–79.0 ug/dl).

4 | DISCUSSION

Endogenous cortisol levels were significantly reduced following treatment with 0.5 mg/kg and 1.0 mg/kg doses of DEX. Cortisol values with lowest dose (0.1 mg/kg) treatment indicated that only one animal was potentially suppressed, while the other three had equivocal or higher post-dose cortisol values. The intermediate (0.5 mg/kg dose) and high (1.0 mg/kg) DEX concentrations lowered cortisol concentrations post-treatment compared with pre-treatment in all animals. All animals displayed reduction in cortisol by at least 30% with the intermediate dose.

In our exploratory analysis of the sex effect, female marmosets had non-significantly higher cortisol levels than male marmosets both pre- and post-dose but only in the low-dose (0.1 mg/kg) group. This was expected as other studies have suggested greater adrenal responsiveness in female marmosets (e.g., marmosets[18], rhesus monkeys[20], and humans.[21]) The most parsimonious explanation for such a sex difference may involve differential gonadal hormone levels, but relationships between sex hormones and cortisol regulation are complex in marmosets.[17] This effect was limited to only one of three groups and these sample sizes for each group were small; thus, we suggest caution in interpreting this result. There was no sex effect within the dose-group (1.0 mg/kg) that significantly suppressed displayed suppression of endogenous cortisol suggesting that this dose could be used in both male marmosets and female marmosets for future DST experimental designs. As only adult marmosets were tested, these doses of DEX should be validated before DST testing in developing and aged marmosets.

In conclusion, we found 0.5 mg/kg and 1.0 mg/kg doses of DEX were effective in reducing endogenous cortisol levels in common marmosets. As individuals' responses to the 0.5 mg/kg dose were substantially more variable than to the higher dose, we suggest this dose be used if the research question concerns individual differences. If one is interested in testing the effect of psychosocial manipulations, then the 1.0 mg/kg may be the better choice.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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