PHARMACOKINETIC AND PHARMACODYNAMIC INTERACTIONS BETWEEN DEHYDROEPIANDROSTERONE AND PREDNISOLONE IN THE RAT

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SUMMARY

The effects of multiple-dosing with dehydroepiandrosterone sulfate (DHEA-SO_4) on the pharmacokinetics and pharmacodynamics of prednisolone were examined. Prednisolone (25 mg/kg i.v.) was administered to male and female Sprague-Dawley rats (250-350 g) alone and following DHEA-SO_4 (4 mg/kg i.v., every 8 h for 4 days). Male control rats cleared prednisolone faster [3.68 ± 1.30 (males) vs 1.01 ± 0.7 l/h/kg; $p<0.05$] and had larger Vss (1.38 ± 0.459 vs 0.394 ± 0.500 l/kg; $p<0.05$) than females both due largely to lesser plasma protein binding. Prednisolone clearance and Vss were not altered by DHEA-SO_4 in males or females. The net effect of prednisolone on basophils and plasma corticosterone did not differ with gender. DHEA-SO_4 had no effect on plasma corticosterone and did not alter prednisolone action. DHEA-SO_4 inhibited basophil trafficking in males, but to a lesser extent than prednisolone, and antagonized the effect of prednisolone on basophil trafficking in both sexes. The steroid-sparing effect observed with DHEA clinically may not be due to an alteration of corticosteroid pharmacokinetics but partly to its ability to affect immune functions.

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KEY WORDS

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INTRODUCTION

Dehydroepiandrosterone (DHEA), a major hormonal precursor, is currently under investigation for treatment of systemic lupus erythematosus (SLE). This is a chronic autoimmune and inflammatory disease that may affect the skin and joints as well as internal organs. Patients with SLE have relatively low levels of DHEA and DHEA-sulfate (DHEA-SO_4). Corticosteroids are prescribed (e.g. prednisone 35 mg/day) for their anti-inflammatory and immunosuppressive actions when SLE symptoms are not well controlled by conservative therapy. Oral DHEA therapy with 200 mg daily doses has been shown to be effective in treatment of SLE and a steroid-sparing effect has been observed /1/.

DHEA is produced by the adrenal glands in response to stimulation by corticotrophin (ACTH). DHEA is uniquely sulfated by an adrenal sulfotransferase to DHEA-SO_4 prior to export into plasma. This inactive metabolite has a relatively long half-life of approximately 10 hours in rats and is continuously hydrolyzed in blood and different organs to produce the free DHEA /2/. In humans, exogenously administered DHEA is metabolized to androstenedione, testosterone and estrogen in the adrenal gland. Androstenedione and testosterone are further metabolized in the liver to 6-hydroxy compounds, metabolites of the CYP3A pathway /3/. DHEA is also metabolized in peripheral tissues to yield androstenediol, androstenetriol, and 7α-hydroxydehydroepiandrosterone. The formation of the latter is stimulated by dexamethasone /4,5/.

Prednisolone is an active metabolite of prednisone and is reversibly converted back to prednisone. The enzyme involved in this interconversion is 11β-hydroxysteroid dehydrogenase (11β-HSDH) which is mainly found in the liver and kidney. Androstenedione, DHEA, and DHEA-SO_4 are competitive inhibitors of 11β-HSDH /6/. Other metabolites of prednisolone include 20-α-(OH)-prednisolone, 20-β-(OH)-prednisolone, and 6-(OH)-prednisolone. The latter is formed by CYP3A isozymes.

Corticosteroids suppress most *in vitro* T-lymphocyte proliferative responses /7-9/ and affect cell trafficking in humans and other species /10,11/. Methylprednisolone has a suppressive effect on basophil trafficking in man which is reflected by decreased whole blood histamine levels /12-14/. Basophils contain 98% of total blood histamine /15/. Exogenous corticosteroids inhibit cortisol secretion by the adrenal gland /12-14/. They also rapidly inhibit basal or circadian release of corticotrophin-releasing hormone (CRH) from the hypothalamus and CRH-stimulated release of ACTH from the pituitary /16-18/. A common side effect of long-term corticosteroid therapy is adrenal suppression /19/.

DHEA *in vitro* suppresses proliferation of murine splenocytes activated by concanavalin A or lipopolysaccharides whereas its metabolite, androstenetriol, potentiates the response to both mitogens /20/. We found synergism between DHEA and prednisolone in the inhibition of rat lymphocyte proliferation *in vitro* /21/.

The involvement of prednisolone and DHEA in the regulation of the immune system, together with their common metabolic pathways may cause pharmacokinetic and/or pharmacodynamic (PK/PD) interactions which may explain the steroid-sparing effect of DHEA. This study was undertaken to assess these possibilities in the rat.

MATERIALS AND METHODS

Animals

Sprague-Dawley male and female rats (Harlan Sprague-Dawley Inc., Indianapolis, IN) weighing 250-350 g were purchased and housed in a 12 hour light/12 hour dark cycle and constant temperature environment (22°C). The rats had free access to rat chow (Agway RMH 1000) and tap water prior to the experiments. A total of 19 rats (8 males and 11 females) were used in the study which adhered to the principles of Laboratory Animal Care (NIH publication # 85-23, revised 1985).

Preparation and sacrifice of animals

A silastic cannula was surgically implanted in the right jugular vein for drug administration and blood sampling. The surgery was done aseptically under ketamine/xylazine anesthesia (50 mg/kg, 10 mg/kg). Cannulas were

kept patent with 100 U/ml of heparin. Animals were sacrificed at the end of the study by removing blood from the aorta after rats were anesthetized.

Drugs

DHEA sodium sulfate (DHEA-SO_4-Na), prednisolone hemisuccinate, prednisone, corticosterone, and betamethasone were purchased from Sigma (St. Louis, MO). A working solution of DHEA-SO_4-Na (10 mg/ml) was made in sterile saline containing 25% ethanol in order to administer a total volume of 425 µl/kg to the animals. Prednisolone hemisuccinate solution (50 mg/ml) was also made in sterile saline such that a total volume of 669 µl/kg was administered to the animals.

Experimental design

The experiment consisted of two parts and was conducted in a quiet room in order to minimize stress to the animals.

Part I - Prednisolone PK/PD in control animals (2-day experiment). Four males and seven females were used. Females were part of a related study assessing the effect of estrus cycle on prednisolone PK/PD. The animals were cannulated one day prior to the experiment. On day 1, starting at noon, blood was collected from the cannula for several time points in order to characterize the baseline dynamics. At noon of day 2, 33.5 mg/kg of prednisolone hemisuccinate (equivalent of 25 mg/kg of prednisolone) was given as an i.v. push in 10 seconds through the cannula followed by frequent blood sampling over 24 hours.

Part II - Effect of multiple dosing with DHEA-SO_4 on prednisolone PK/PD (5-day experiment). Four males and four females were used. The animals were cannulated under sterile conditions one day prior to treatment. The next day, the rats were dosed with 4.25 mg/kg of DHEA-SO_4-Na (equivalent of 4 mg/kg of DHEA-SO_4) as an i.v. push in 10 seconds through the cannula starting from 4:00 p.m. The dosing was repeated every eight hours for a total of 9 doses. Baseline dynamics were characterized on day 4 starting at noon. On day 5, the animals were dosed with 4 mg/kg equivalent of DHEA-SO_4 15 minutes before 25 mg/kg of i.v. prednisolone. Blood was then drawn serially.

Blood sampling schedule

For the baseline phases, 0.6 ml of blood were drawn by a heparinized syringe at time 0, 2, 4, 6, 8, 10, 12, and 24 hours. After each blood sample,

the cannulas were flushed with 0.6 ml of sterile saline. Fifty µl of heparinized blood were lysed with 950 µl of double distilled water and kept at -20°C for later determination of whole blood histamine. The remaining blood was centrifuged for 10 minutes at 10,000 x *g*. The plasma was then aliquoted into an Eppendorf tube and stored at -20°C.

For determination of prednisolone PK/PD, blood was taken at time 0, 0.16, 0.33, 0.50, 0.75, 1, 2, 3, 4, 6, 8, 10, 12, and 24 hours. Over 0 to 1 hour, only 0.3 ml of blood was drawn and replaced with 0.3 ml of sterile saline. For remaining samples, 0.6 ml of blood was taken and 0.6 ml of sterile saline was replaced. The blood samples were treated as before.

Assay methodology

HPLC

Prednisone, prednisolone and corticosterone were measured by the HPLC method of Rose and Jusko /22/ adapted for samples from rats /25/. Chromatography was performed on a DuPont Zorbax Sil column using a mobile phase of methylene chloride-heptane-ethanol-acetic acid (600:350:40:10) at a flow rate of 2.0 ml/min. The lower limit of quantitation (LOQ) was 10 ng/ml for all three drugs. The interday coefficients of variation (CV) for prednisone were 3.11% at 20 ng/ml, 2.65% at 75 ng/ml, and 2.85% at 200 ng/ml. The interday CV for prednisolone were 7.03% at 25 ng/ml, 2.70% at 150 ng/ml, and 2.76% at 600 ng/ml. Interday CV for corticosterone were 7.23% at 20 ng/ml, 4.10% at 75 ng/ml, and 5.09% at 200 ng/ml. No assay interferences were found after administration of DHEA-SO_4.

Plasma protein binding

Plasma protein binding of prednislone was assessed by ultrafiltration using ^{3}H-prednisolone (68.8 Ci/mmol) which was purified by HPLC. Plasma from male and female controls was spiked with prednisolone in concentrations ranging from 42 to 20,000 ng/ml. A 25-µl aliquot (15,000 dpm) of ^{3}H-prednisolone was added to 0.650 ml of plasma samples. The samples were loaded onto Centrifree devices purchased from Amicon (Beverly, MA) and then centrifuged (1,000 x *g* for 30 min at 37°C). After centrifugation, the cpm of buffer and plasma samples were converted to dpm using the sample channel ratio technique to obtain:

$$\text{Fraction free} = (\text{dpm/ml ultrafiltrate})/(\text{dpm/ml plasma})$$

Whole blood histamine

Whole blood histamine was analyzed by radioimmunoassay (Immunotech International, Westbrook, ME). The upper and lower limits of quantitation were 11.1 and 0.111 ng/ml. The CV for the standards were 5.69% at 0.111 ng/ml and 10.7% at 3.33 ng/ml.

Data analysis

Pharmacokinetics

The plasma concentrations of prednisolone (Cc) were fitted to differential equations using PCNONLIN (SCI Software, Inc., Apex, NC) with 1/Cc weighting. The prednisolone dose was assumed to be an i.v. bolus.

The equations used were:

$$\frac{dCc}{dt} = \frac{CL_D \cdot A_T / V_T - (CL_D + CL_E) \cdot Cc}{V_C} \quad (1)$$

$$\frac{dC_T}{dt} = \frac{CL_D \cdot Cc - CL_D \cdot A_T / V_T}{V_T} \quad (2)$$

where C_T and A_T are prednisolone concentration and amount in the tissue compartment, V_C and V_T are the volumes of central and tissue compartments, CL_D is the distribution clearance, and CL_E is the elimination clearance. The volume of distribution at steady-state ($V_{SS} = V_c + V_T$), the mean residence time ($MRT = V_{SS}/CL_E$), the hybrid rate constants α and β, and half life ($t1/2_\beta = 0.693/\beta$) were calculated as secondary parameters. The areas under the concentration-versus-time curve extrapolated to infinity of prednisone (AUC_{PN}) and prednisolone (AUC_{PD}) were calculated using the Spline method.

Pharmacodynamics

To characterize the overall effects of prednisolone on corticosterone secretion and basophil trafficking (whole blood histamine), the area between the baseline and effect curves (ABEC) and the area suppression ratio (AUC_{SR}) were used. These were determined by:

$$ABEC = AUC_0^{12}{}_{BL} - AUC_0^{12}{}_{E} \quad (3)$$

$$AUCSR = \frac{AUC_0^{12}{}_E}{AUC_0^{12}{}_{BL}} \quad (4)$$

where $AUC_0{}^{12}{}_E$ is the area under the effect curves from 0 to 12 hours, and $AUC_0{}^{12}{}_{BL}$ is the area under the baseline curves from 0 to 12 hours. Corticosterone concentrations below the LOQ were assigned the value of 5 ng/ml (½ the LOQ) for determination of ABEC and AUC_{SR}.

Statistical analysis

Prednisolone PK/PD parameters in male and female control rats were assessed by ANOVA. The same statistical analysis was used to examine the effect of multiple dosing with DHEA-SO_4 on prednisolone PK/PD. ABEC and AUC_{SR} were not calculated after multiple-dosing of DHEA-SO_4 for basophil trafficking. The gender effect for these two parameters under prednisolone alone was otherwise assessed by Student's unpaired t-test. Repeated measures ANOVA was used to test the differences of baseline whole blood histamine in animals dosed with and without DHEA. All tests were set at a statistical significance of $P<0.05$ using INSTAT (GraphPad Software, La Jolla, CA).

RESULTS

Pharmacokinetics

Figure 1 shows representative prednisolone and prednisone plasma concentration-versus-time profiles for male and female rats given single doses of prednisolone alone and after multiple-dosing with DHEA-SO_4. The hydrolysis of prednisolone sodium hemisuccinate to prednisolone was rapid since no prodrug was detected as early as 10 minutes post-injection. No interfering peak was detected for prednisolone hemisuccinate from the chromatograms. The disposition of prednisolone is biexponential with a rapid initial phase followed by a slower elimination phase. Prednisone was rapidly formed from prednisolone and its terminal slope was parallel to that of the parent drug. The AUC_{PN} is considerably smaller (3 to 5%) than AUC_{PD}.

Figure 2 illustrates the protein binding profile for male and female control rats as a function of plasma prednisolone concentration. All

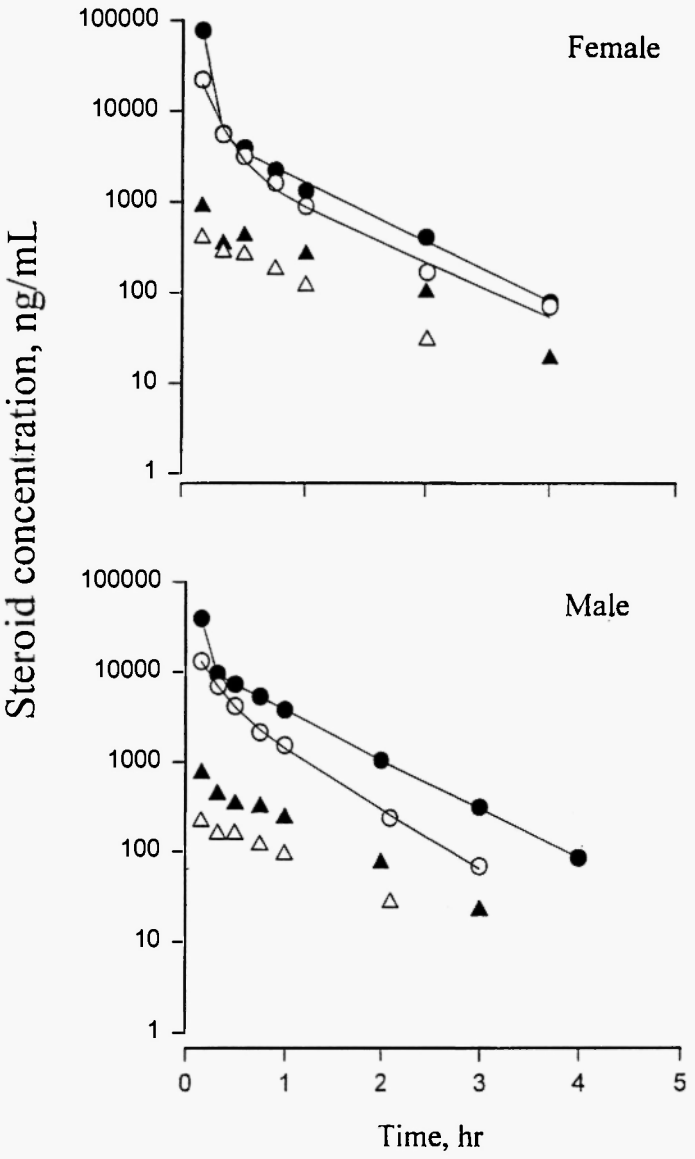

Fig. 1: Representative prednisone and prednisolone plasma concentration versus time profiles for male and female rats administered single-dose prednisolone alone and after multiple dosing with DHEA-SO_4. Symbols show experimental data and lines represent the fittings to eq. 1 and 2. Key: ● Prednisolone, O Prednisolone + DHEA, ▲ Prednisone, Δ Prednisone + DHEA.

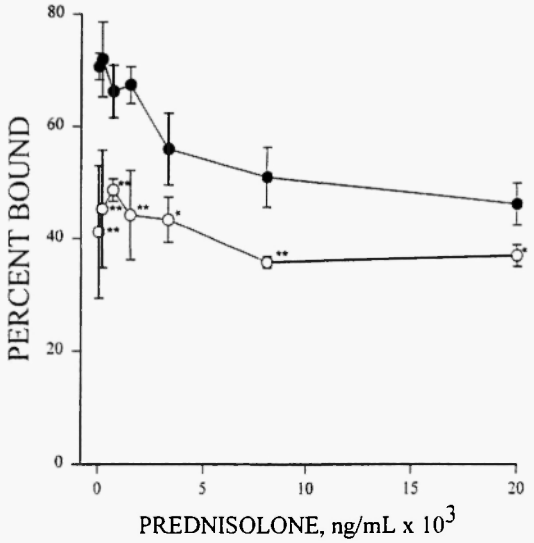

Fig. 2: Percent of plasma protein binding of prednisolone as a function of concentration in male and female rats. Data are mean ± SD of 4 females (●) and 3 males (O). * $P<0.05$, ** $P<0.01$

profiles exhibited nonlinearity consistent with saturation in transcortin binding and linear binding to albumin. Females exhibited greater percent of plasma protein binding of prednisolone than males.

Prednisolone pharmacokinetic parameters when given with and without DHEA are summarized in Table 1. Male controls had higher CL_E than female rats (3.68 ± 1.30 versus 1.01 ± 0.78 l/h/kg: $p < 0.01$). The CL_D, V_T, and V_{SS} were also larger in males. There were no significant differences in other pharmacokinetic parameters between males and females. Gender differences were no longer observed in the pharmacokinetics of prednisolone after dosing with DHEA-SO_4.

Pharmacodynamics

Corticosterone suppression

Figure 3 shows typical plasma corticosterone concentration-versus-time profiles in male and female rats at baseline and following dosage with prednisolone with and without DHEA-SO_4. After prednisolone

TABLE 1

Pharmacokinetic parameters for prednisolone in male and female rats

Parameter	Prednisolone Alone			Prednisolone + DHEA	
	Males (n = 4)	Females (n = 7)	P value	Males (n = 4)	Females (n = 7)
CL_E (L/h/kg)	3.68 ± 1.30	1.01 ± 0.78	< 0.05	2.51 ± 1.43	0.685 ± 0.474
CL_D (L/h/kg)	1.35 ± 0.64	0.202 ± 0.294	< 0.05	0.728 ± 0.422	0.161 ± 0.271
V_c (L/kg)	0.879 ± 0.426	0.264 ± 0.366	NS	0.584 ± 0.516	0.445 ± 0.763
V_T (L/kg)	0.500 ± 0.179	0.128 ± 0.180	<0.05	0.381 ± 0.206	0.125 ± 0.141
V_{SS} (L/kg)	1.38 ± 0.46	0.394 ± 0.500	<0.05	0.969 ± 0.704	0.140 ± 0.224
α (h^{-1})	7.60 ± 4.11	13.2 ± 8.65	NS	10.2 ± 6.66	18.4 ± 4.42
β (h^{-1})	1.64 ± 0.34	1.39 ± 0.35	NS	1.42 ± 0.43	1.44 ± 0.18
$t_{1/2}$ (h)	0.435 ± 0.100	0.528 ± 0.169	NS	0.523 ± 0.161	0.484 ± 0.053
MRT (h)	1.11 ± 0.44	1.15 ± 0.80	NS	1.31 ± 0.610	0.565 ± 0.380
AUC_{PN}/AUC_{PD}	0.0360 ± 0.0071	0.0525 ± 0.0180	NS	0.0349 ± 0.0075	0.0490 ± 0.0320

Data are means ± S.D. PN (prednisone), PD (prednisolone). No statistically significant differences were observed in the pharmacokinetic parameters of prednisolone within genders between the prednisolone and the prednisolone + DHEA groups.

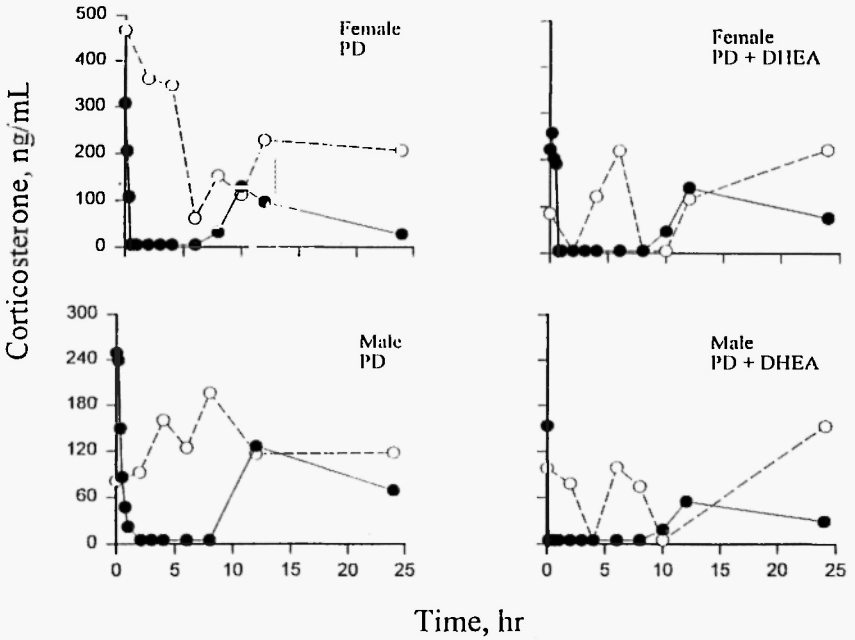

Fig. 3: Representative plasma corticosterone concentration versus time profiles for a female and male rat dosed with prednisolone (25 mg/kg i.v.) alone and with DHEA-S. Key: O Baseline phase, ● Prednisolone phase.

administration, plasma corticosterone dropped abruptly during the first two hours and remained below the limit of quantitation for up to 8 hours post-dosing. In both genders, baseline levels of corticosterone were recovered about 12 hours following the prednisolone dose. The dynamic data for corticosterone suppression in male and female rats are listed in Table 2. There was large variability in baseline corticosterone concentrations at 0, 6, 12, and 24 h between rats in different groups. Despite this large variability, a general pattern for corticosterone secretion was observed. Baseline concentrations were high at 12:00 noon and low at 6:00 p.m. and 12:00 midnight. The concentrations returned to similar levels at noon the next day, reflecting a circadian rhythm. The same trend was seen after DHEA dosing in male

TABLE 2

Pharmacodynamic parameters for corticosterone suppression

Parameter	Prednisolone		Prednisolone + DHEA	
	Males (n =4)	Females (n = 7)	Males (n = 4)	Females (n =4)
C_0[a]	130 ± 67	182 ± 163	44.7 ± 57.7	97.4 ± 98.6
C_6[a]	80.4 ± 51.6	103 ± 30.0	34.9 ± 23.9	191 ± 74.6
C_{12}[a]	88.9 ± 32.2	109 ± 146	21.4 ± 17.5	73.7 ± 60.2
C_{24}[a]	115 ± 96.5	170 ± 91.0	96.9 ± 33.8	132 ± 78.4
$AUC_0^{12}{}_{BL}$ (ng ·hr/ml)	983 ± 469	1620 ± 725	972 ± 335	1370 ± 510
$AUC_0^{12}{}_{E}$ (ng·hr/ml)	323 ± 50	401 ± 237	296 ± 243	311 ± 166
ABEC (ng·hr/ml)	760 ± 286	660 ± 467	1169 ± 660	650 ± 158
AUC_{SR}	0.38 ± 0.19	0.44 ± 0.17	0.24 ± 0.13	0.23 ± 0.16

[a] Baseline concentrations (ng/ml) at serial times (hr); No statistically significant differences were observed in AUC, ABEC and AUC_{SR} between and within genders in the prednisolone and prednisolone + DHEA groups. Data are means ± S.D.

rats but not in the female rats. The inhibition of corticosterone secretion after coadministration of prednisolone and DHEA-SO_4 followed the same pattern as that seen in control animals. The areas under the baseline curves were similar after multiple-dosing with DHEA-SO_4 in male and female rats. Prednisolone effects on corticosterone were assessed by ABEC and AUC_{SR}. No changes in these parameters were observed after coadministration of prednisolone and DHEA-SO_4 within or between genders (Table 2).

Basophil trafficking

Whole blood histamine concentrations were relatively stable during the saline baseline in male rats. Complete baseline basophil trafficking was characterized in control male rats and in females and males dosed with DHEA-SO_4. Since the female controls were part of a related study investigating the effect of the estrus cycle on prednisolone PK/PD, whole blood histamine concentrations were measured only at 0 and 24 hours in female controls. This was done to assure that baseline and PD phases were studied at the same stage of the estrus cycle. Indeed, the rat estrus cycle comprises four different phases lasting 24 hours each which exhibit different levels of sex hormones /23/. Basophil trafficking does not follow a circadian rhythm /12-14/, and thus histamine concentrations at 0 and 24 hour at baseline for this group were taken as the average of the two measured values.

Whole blood histamine concentrations declined following prednisolone administration to the control groups. The nadir was reached at 8 to 12 hours post-dosing and the initial concentrations were recovered after 24 hours. Males had higher concentrations than female rats (Fig. 4). The data for corticosterone suppression are shown in Table 3. The ABEC was significantly greater in males than females. However, no gender differences were observed for AUC_{SR}.

The baseline histamine concentrations after multiple-dosing with DHEA-SO_4 were stable over 24 hours and were lower than that of male control rats (Fig. 5). DHEA produced an inhibitory effect on basophil trafficking in male rats, but this was less marked than for prednisolone. This effect of DHEA-SO_4 was less obvious in female rats. The inhibitory effect of prednisolone on basophil trafficking observed in control animals was not seen after concomitant administration of prednisolone and DHEA-SO_4. DHEA-SO_4 antagonized the effects of prednisolone on basophil trafficking. In male rats basophil trafficking followed an unusual pattern after joint dosing of the two drugs (Fig. 4) with a rise in whole blood histamine above baseline levels for up to 8 hours followed by an appreciable decline. Baseline levels were still not recovered by 24 hours post dosing. Because of the complex effect of DHEA-SO_4 on prednisolone action, ABEC and AUC_{SR} could not be measured.

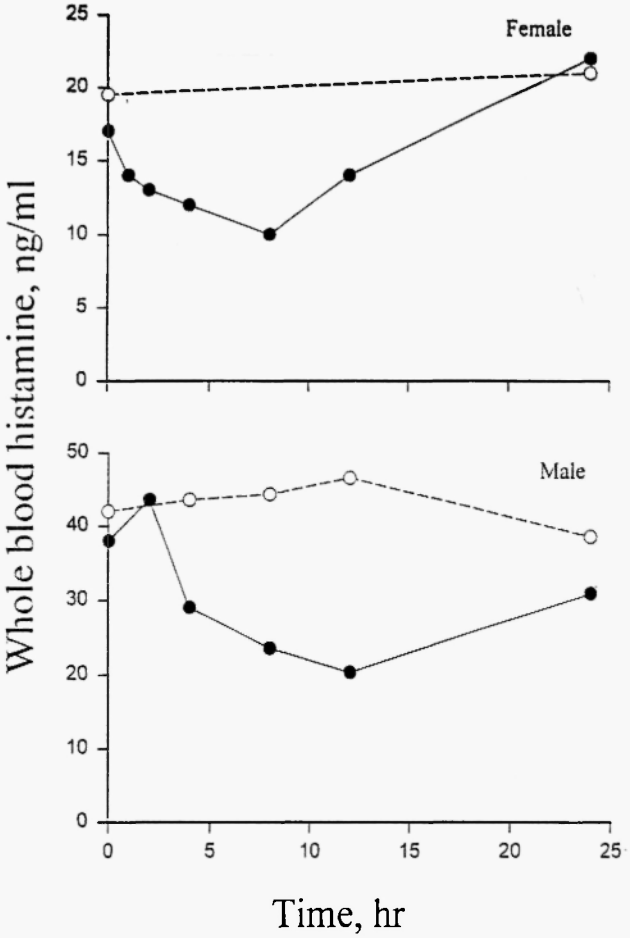

Fig. 4: Representative plasma whole blood histamine concentration versus time profiles for a female and a male rat dosed with prednisolone. Key: O Baseline phase, ● Prednisolone phase.

DISCUSSION

Pharmacokinetics

Prednisolone is reversibly metabolized to prednisone. The elimination of prednisone is governed by this reversibility as suggested by its terminal slope which parallels that of prednisolone. Calculation of the

TABLE 3

Pharmacodynamic parameters for basophil trafficking in male and female rats

Parameter	Prednisolone		
	Males (n =4)	Females (n = 7)	P value
$AUC_0^{12}{}_{BL}$ (ng·hr/ml)	508 ± 67	294 ± 75	< 0.05
$AUC_0^{12}{}_{E}$ (ng·hr/ml)	316 ± 45	198 ± 78	< 0.05
ABEC (ng·hr/ml)	191 ± 34	96.4 ± 26.1	< 0.05
AUC_{SR}	0.623 ± 0.042	0.661 ± 0.113	NS
$C_{0,BL}$[a]	45.8 ± 3.2	24.5 ± 6.2	< 0.05

ABEC and AUC_{SR} are defined in the text. [a] Baseline whole blood histamine concentrations (ng/ml) at 12: 00 noon. NS, not significant. Data are means ± S.D.

true pharmacokinetic parameters of prednisolone requires i.v. administration and measurement of both drug and metabolite /24/. Therefore, the pharmacokinetic parameters reported herein are apparent, but prednisone is only a small fraction (3-5%) of prednisolone concentrations. The CL_E of prednisolone observed in our study is consistent with values obtained previously /25/. A higher CL_E and CL_D were found in male compared to female rats.

Prednisolone is metabolized in the liver to a 6 hydroxy-metabolite which is formed by CYP3A enzymes. This metabolite accounts for 18% of the total excreted metabolites in man /26/. The lower clearance in female rats could be explained by lower CYP3A activity in female rats /27/. Secondly, CYP3A catalyzes the formation of 2-(OH)-estradiol-17β, a major metabolite of estradiol-17β /28/. Also, there was greater plasma protein binding of prednisolone in females.

Female rats also had smaller V_T and V_{SS} compared to males. We observed higher plasma protein binding of prednisolone in female rats.

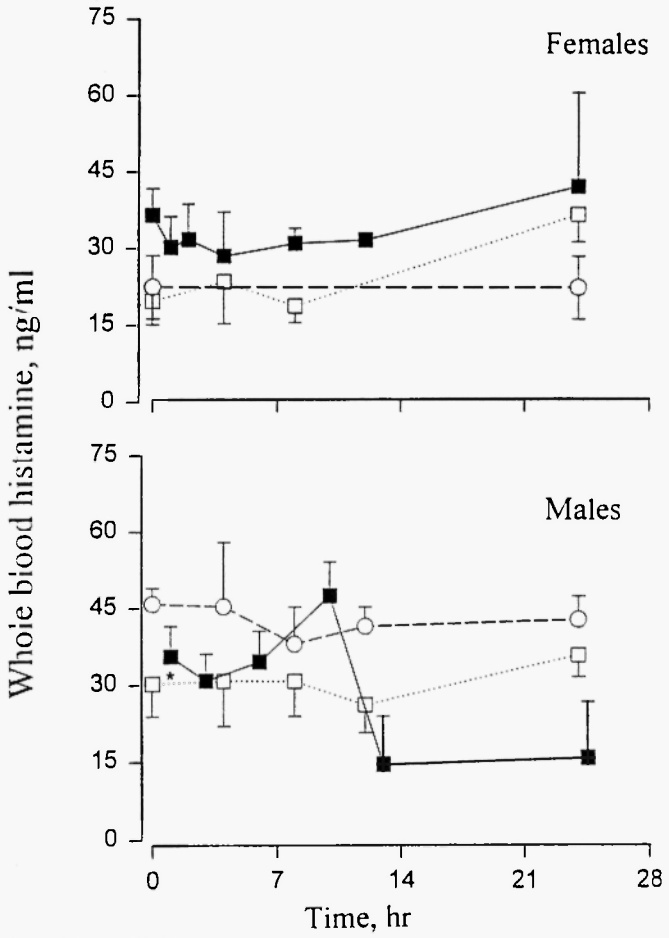

Fig. 5: Basophil trafficking patterns in male and female rats given single-dose prednisolone (25 mg/kg) following multiple dosing with DHEA-SO_4. Symbols represent mean ± S.D. of 4 animals. Key: O Baseline saline, □ DHEA baseline, ■ Prednisolone + DHEA. * $P < 0.05$ versus baseline saline.

Estrogens stimulate the production of corticosteroid binding globulin thus increasing binding of prednisolone /29/. Prednisolone $t_{1/2}$ was not different between sexes. This is consistent with a joint change in clearance and volume in male rats.

Dosing with DHEA-SO_4 did not alter prednisolone pharmacokinetics in either sex. Contrary to our initial hypothesis, the effects of DHEA-SO_4 on different enzymes involved in the metabolism of corticosteroids may be insignificant or cancel each other out. However, gender differences in prednisolone pharmacokinetics were not observed after coadministration with DHEA-SO_4.

Pharmacodynamics

Corticosterone suppression

Corticosteroids are used for their immunosuppressive effects in various disease states. They are often combined with other drugs with similar therapeutic effects such that lower doses of corticosteroids can be used. Even though adrenal gland functionality is usually assessed by the ACTH stimulation test /30/, cortisol or corticosterone inhibition may indicate long-term suppression. The assessment of corticosterone secretion relies on the correct measurement of baseline levels. We managed to markedly reduce stress to the animals by working in a quiet room. Our corticosterone results are consistent with literature values /31/.

The production of DHEA from the adrenal gland is stimulated by ACTH. DHEA-SO_4 was used in our study as an inactive prodrug at doses per kilogram of body weight comparable to those used in clinical studies /1/. We assume that a pseudo steady-state was reached with this dosage regimen since DHEA-SO_4 was administered over 12 half-lives. We expected that exogenous DHEA could suppress ACTH which regulates corticosterone secretion by the adrenal gland. Four-day administration of DHEA-SO_4 to rats did not affect baseline corticosterone secretion in male and female rats. The ABEC and AUC_{SR} were not significantly different with or without DHEA-SO_4 or when the two drugs were administered. We therefore speculate that this effect of prednisolone may not be exacerbated when the corticosteroid is combined with DHEA.

Basophil trafficking

Lew *et al.* /32/ found higher whole blood histamine ABEC in men compared to women without differences in AUC_{SR}. These gender differences were mimicked in our study. The inhibition of basophil trafficking by prednisolone was counterbalanced by DHEA-SO_4 in

both sexes. Basophils, like other immune cells, traffic continuously between the blood and extravascular compartments. Adhesion molecules regulate the movement of immune cells through the endothelium /33/. IL-3 and GM-CSF induce basophil chemotaxis /34/. The pharmacodynamic effect of steroids on basophil trafficking was modeled by Wald *et al.* /14/. The first-order rate constant for egress from the blood compartment of some immune cells (basophils, helper-T cells) is not affected by corticosteroids. In contrast, basophils are sequestered in the peripheral tissues and a zero-order rate constant describes their return to the blood. The interaction of DHEA-SO_4 with prednisolone observed in this study may occur in the regulation of either adhesion molecules or IL-3 synthesis. Any effect of DHEA-SO_4 on adhesion molecules is yet to be documented, but DHEA depresses IL-3 production from ConA-activated mice lymphocytes while its metabolite produces opposite effects /20/. The involvement of active metabolites of DHEA may further complicate the interpretation of our observations.

From the results of the current study, it appears that any steroid-sparing effect of DHEA may be due to its intrinsic effects on certain immune responses rather than alteration of corticosteroid disposition.

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