Influence of Gender on Prednisolone Effects on Whole Blood T-Cell Deactivation and Trafficking in Rats

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Abstract □ Prednisolone (5 mg/kg intravenous) was administered to adrenalectomized male and female Sprague-Dawley rats (250–350 g) to assess the effects of gender on disposition and pharmacoimmunodynamics. Plasma concentrations of prednisolone were determined by high-performance liquid chromatography. Incorporation of [³H]thymidine (³H-TDR) was used to determine whole blood T-cell (WBTC) trafficking and deactivation following stimulation with Concanavalin-A. Whole blood T-cell trafficking was determined indirectly by using the glucocorticoid receptor antagonist RU-40555 (250 ng/mL) added to ex vivo cultures of whole blood from animals dosed with prednisolone. Mean (± SD) prednisolone clearance values were 3.22 ± 0.88 and 3.46 ± 0.96 L/h/kg in males and females, respectively. After administration of prednisolone, relative T-cell counts decreased slowly with time to reach a nadir at 3–5 h and returned to baseline levels by 8 h. Fitting data using an indirect response model yielded mean prednisolone 50% inhibitory concentration for inhibition of WBTC trafficking (IC_{50T}) that was lower in males compared with females (0.14 ± 0.16 versus 1.03 ± 0.06 ng/mL; $p < 0.05$). In the absence of RU-40555, an immediate and complete inhibition of ³H-TDR incorporation into WBTC was observed (deactivation) and baseline levels were recovered slowly as prednisolone was cleared from blood. The mean 50% inhibitory concentration for inhibition of WBTC deactivation (IC_{50D}) based on an inhibitory I_{max} model was similar in males and females (0.20 ± 0.24 versus 0.18 ± 0.12 ng/mL). Although male and female rats have similar exposure to prednisolone after 5-mg/kg doses, males are more sensitive to the inhibition of WBTC trafficking, whereas no gender effects on deactivation of WBTC exist.

Introduction

Glucocorticoids have a major role in the treatment of allograft rejection and several allergic, autoimmune, and malignant diseases in humans.[1] They profoundly suppress various nonspecific inflammatory responses and specific immunologic processes.[2] The mechanism by which they exert these actions is yet to be fully understood. However, lymphocytes mediate several glucocorticoid responses. The importance of trafficking of circulating lymphocytes and monocytes in mediating corticosteroid action has been documented.[3] In addition, corticosteroids profoundly inhibit T-cell activation (clonal expansion of antigen specific T-cells), cellular events that occur during an encounter with peptide antigens. Some of the current knowledge of cellular events in T-cell activation is based on in vitro experiments in which T-cells can be stimulated in a controlled manner with lectins, such as concanavalin-A (Con-A), and their proliferation can be measured by the incorporation of [³H]-thymidine (³H-TDR).[4]

Considering heart transplant recipients treated with prednisone and cyclosporine, female recipients have more rejection episodes at 3 and 12 months than do males. Also, early withdrawal of maintenance steroids is less often achievable in women as compared with men (17 versus 65%).[5] These differences may be explained by genetic differences between genders and/or by the interactions of sex hormones with glucocorticoids in the regulation of the immune system. Indeed, estrogens stimulate the production of corticosteroid binding globulin (CBG) in rats.[6] This stimulation may alter the kinetics of prednisolone.[7] The influence of female sex hormones on glucocorticoid receptor binding parameters has also been observed in some responsive organs.[8,9] Lew et al.[10] found a correlation between the logarithm of plasma estradiol-17β and sensitivity of women to methylprednisolone inhibition of basophil trafficking. Moreover, estradiol inhibits rat thymocyte proliferation, T-cell suppressor activity, and mixed lymphocyte reactions.[11−13]

Improved use of glucocorticoids as antiinflammatory or immunosuppressive agents may be achieved by an understanding of the influence of covariates, such as gender, in their pharmacokinetics (PK) and pharmacodynamics (PD). The objectives of this work were to assess the influence of gender on prednisolone pharmacokinetics in conjunction with gender influences on prednisolone effects on whole blood T-cell (WBTC) trafficking and deactivation in rats.

Experimental Section

Animals—Adrenalectomized Sprague-Dawley male and female rats weighing 250−350 g were purchased from Harlan Sprague-Dawley Inc. (Indianapolis, IN) and housed in a 12 h light/12 h dark cycle and constant temperature environment (22 °C). The rats had free access to rat chow (Agway RMH 1000) and normal saline (NaCl, 0.9%) prior to the experiments. A total of 8 rats (4 males and 4 females) were used in the study, which adhered to the principles of Laboratory Animal Care (NIH publication # 85-23, revised 1985). Female rats were taken at proestrus (phase of the ovarian cycle exhibiting high levels of sex hormones). The rat estrus cycle lasts 4−5 days and is identifiable by changes in the vaginal cytology. Each phase exhibits different levels of sex hormones.[14]

A silastic cannula was surgically implanted in the right jugular vein for drug administration and blood sampling 7 days prior to study day. The surgery was done aseptically under ketamine/xylazine anesthesia (50/10 mg/kg). Cannulas were flushed daily and kept patent with 200 U/mL of heparin.

Materials—Prednisolone hemisuccinate and betamethasone were purchased from Sigma (St. Louis, MO). A working solution of prednisolone hemisuccinate at 13.4 mg/mL (equivalent of 10 mg/mL prednisolone) was made in sterile saline to administer a total volume of 500 μL/kg to the animals. RU-40555 was obtained as a gift from Roussel Uclaf (Romainville, France). Tritiated thymidine (³H-TDR; 6.7 Ci/mmol) was purchased from Amersham

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(Arlington Heights, IL). Concanavalin-A (Con-A) was obtained from ICN Biomedicals (Cleveland, OH).

Experimental Design—For assessment of incorporation of ^{3}H-TDR into WBTC (relative cell counts) as a function of time at baseline, 0.3 mL of blood were drawn with a heparinized syringe at 0, 3, 6, 9, and 12 h. Each blood sample was replaced with 0.3 mL of sterile normal saline. Because each phase of a 4-day estrus cycle lasts only 24 h, prednisolone was administered 4 days after baseline measurements. This delay allowed measurement of T-cell counts during baseline and prednisolone phases at the same stage of the estrus cycle of female rats. Prednisolone hemisuccinate (equivalent of 5 mg/kg of prednisolone) was administered as an intravenous (iv) bolus through the cannula, and then blood was sampled frequently over 12 h. After prednisolone administration, 0.4 mL of blood was withdrawn at 0.16, 0.33, 0.5, 0.75, 1, 2, 3, and 4 h and 0.2 mL at 0, 5, 6, 8, 9, and 12 h. Each blood sample was replaced with an equivalent volume of sterile normal saline. One hundred microliters of heparinized blood were used for ex vivo whole blood cultures. The remaining blood was centrifuged for 10 min at 5000 x g. The plasma was then aliquoted into Eppendorf tubes and stored at -20 °C for assay of prednisolone concentrations.

Assay Methodology—*Whole Blood Cultures*—The method of Fasanmade and Jusko[15] was used. Blood diluted 15-fold (145 μL) was dispensed into each well of 96-well flat-bottomed polystyrene multiple-well plate. Con-A (20 μL) was added to culture medium to give a final concentration of 10 ng/mL. Supplemented RPMI 1640 was added to give a total volume of 200 μL per well. Cells were incubated at 37 °C under a humidified atmosphere of 7% CO_2 for 96 h before adding ^{3}H-TDR (1 μCi per well). Cells were harvested with an automatic harvester (Skatron Instruments, Sterling, VA) at 20 h after the addition of ^{3}H-TDR. Cellularly incorporated radioactivity was assessed using a Packard Topcount microplate scintillation counter (Downers Grove, IL).

In Vitro Interaction of RU-40555 with Prednisolone in WBTC Deactivation—To determine the competitive inhibition of glucocorticoid receptors in vitro, whole blood from male rats was spiked with prednisolone to obtain final concentrations of 200, 1000, 2000, and 5000 ng/mL and incubated for 2 h at 37 °C prior to adding RU-40555 (250 ng/mL). The optimum concentration of RU-40555 was determined by Fasanmade and Jusko.[16] Whole blood was then diluted and stimulated with Con-A and cultured for an additional 96 h as described in the previous section. RU-40555 produces a complete inhibition of glucocorticoid receptor when the molar ratio RU-40555/glucocorticoids is 10 or greater. There is no difference between genders regarding the molar ratio for optimum inhibition (Dr. Gaillard-Kelly, personal communication, Roussel Uclaf).

High-Performance Liquid Chromatography (HPLC)—Prednisone was measured by the HPLC method of Rose and Jusko[17] as adapted for rats[18,19] using betamethasone as internal standard. The lower and upper limits of quantitation were 10 and 1500 ng/mL. The interday coefficient of variation (CV) for prednisolone was 7.03% at 25 ng/mL, 2.70% at 150 ng/mL, and 2.76% at 600 ng/mL.

Data Analysis—*Pharmacokinetics*—Prednisolone plasma concentrations (C_p) versus time (t) were described by:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} \qquad (1)$$

The intercept coefficients (A and B) and slopes (α and β) were estimated by least-squares fitting using the ADAPT II.4 program (Biomedical Simulation Resource, Los Angeles, CA). The area under the prednisolone concentration–time curve (AUC = A/α + B/β) and the area under the first-moment curve (AUMC = A/α^2 + B/β^2) were then calculated. The mean residence time (MRT) was determined as the ratio of AUMC/AUC. Clearance (CL) was obtained as dose/AUC. The volume of distribution at steady-state was determined as CL·MRT.

Pharmacodynamics—The model describing the time course of lymphocyte and ex vivo mitogen activation is depicted in Figure 1.

Lymphocyte Trafficking—Incorporation of ^{3}H-TDR into WBTC was used as an indirect measurement of WBTC counts. Because prednisolone inhibits lymphocyte trafficking, the incorporation of ^{3}H-TDR into WBTC varied with time following the pattern of a typical indirect response. Because blood samples from animals dosed with prednisolone contained the drug, complete deactivation
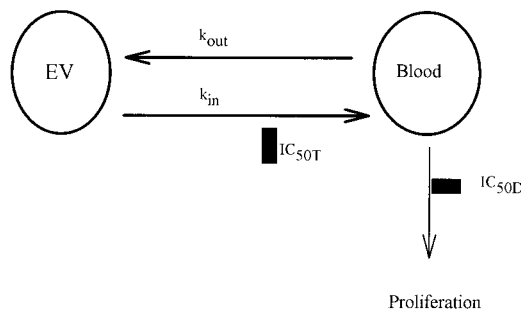

Figure 1—Pharmacodynamic model for the effects of prednisolone on whole blood T-cell trafficking and deactivation. Cells egress from blood to extravascular (EV) tissues with rate constant k_{out}, and the rate constant for return to blood is k_{in}. The IC_{50T} reflects prednisolone inhibition of k_{in} and IC_{50D} reflects the inhibition of mitogen-stimulated activation of whole blood T-cells.

of lymphocytes was observed after stimulation with Con-A causing inhibition of ^{3}H-TDR incorporation into WBTC. Thus, to assess the relative number of T-cells in 100 μL of diluted whole blood at each time point, samples of diluted blood from dosed animals were incubated with the glucocorticoid receptor antagonist RU-40555 (250 ng/mL). Complete blockade of prednisolone action then allowed the incorporation of ^{3}H-TDR into lymphocytes present in whole blood samples.

Blood lymphocytes (L) circulate between the central and extravascular compartments. Corticosteroids inhibit the zero-order rate (k_{in}) of return of lymphocytes from the extravascular compartment without affecting their movement out of the central blood compartment (k_{out}). The scheme in Figure 1 and the following equation describe lymphocyte trafficking:[20]

$$\frac{dL}{dt} = k_{in} - k_{out}L \qquad (2)$$

in which $L = L_0$ (cpm pre-dose) at $t = 0$. In the presence of prednisolone, the k_{in} is inhibited and the rate of change of lymphocyte counts with time is described by

$$\frac{dL}{dt} = k_{in}\left(1 - \frac{I_{max}C_P}{IC_{50T} + C_P}\right) - k_{out}L \qquad (3)$$

where C_p is prednisolone plasma concentration, IC_{50T} is the concentration of prednisolone producing half-maximal inhibition (I_{max}) of k_{in}, and L is the observed cpm in ex vivo cultures treated with RU-40555.

Lymphocyte Deactivation—When whole blood samples drawn at various time points after prednisolone administration were cultured, an immediate and complete inhibition of ^{3}H-TDR incorporation into lymphocytes was observed and baseline levels recovered gradually. This inhibition was due to prednisolone concentrations present in blood samples. The level of ^{3}H-TDR observed was a function of the number of cells present in the blood at a given time (trafficking) and also a function of prednisolone plasma concentrations.

Lymphocyte deactivation by prednisolone is described as follows by the I_{max} model:

$$E = \left(1 - \frac{I_{max}C_P}{IC_{50D} + C_P}\right)L \qquad (4)$$

where E is the observed cpm in cells from blood samples not treated with RU-40555 (intrinsic deactivation) and L is the observed cpm in cells treated with RU-40555, C_p is prednisolone plasma concentrations corrected for the dilution of blood, and IC_{50D} is prednisolone concentration producing half-maximal inhibition (I_{max}) of lymphocyte activation.

When both inhibition of cell trafficking and deactivation of lymphocytes occur, eqs 3 and 4 are fitted simultaneously.

To assess the net deactivation of WBTC following prednisolone administration, the area under lymphocyte trafficking (baseline for the deactivation process) and deactivation curves from 0 to 12 h were calculated using the Spline method.[21] These values were used to calculate the area between baseline and effect curves (ABEC) and the area suppression ratio (AUC$_{SR}$) determined by
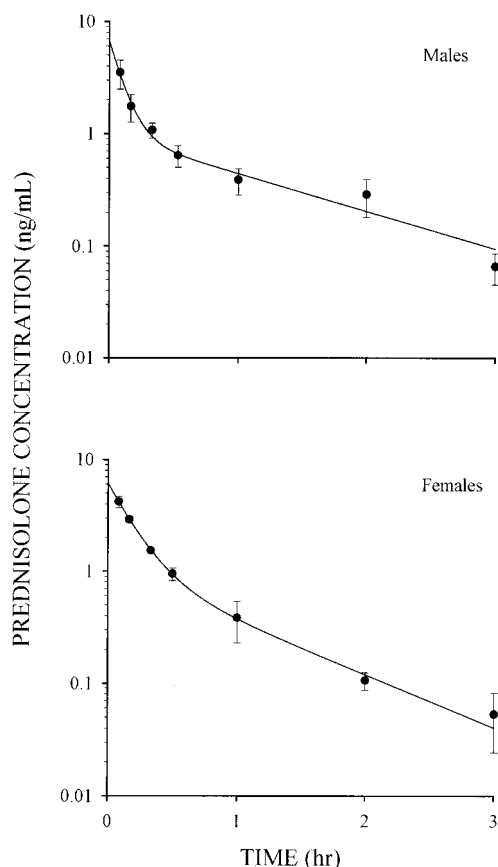
Figure 2—Mean (± SD) plasma concentration versus time profiles of prednisolone for males and females after iv administration of prednisolone 5 mg/kg. Symbols represent experimental data and lines are least-squares regression fittings.

eqs 5 and 6

$$\text{ABEC} = \text{AUC}_{0\ \text{BL}}^{12} - \text{AUC}_{0\ \text{E}}^{12} \tag{5}$$

$$\text{AUC}_{\text{SR}} = \frac{\text{AUC}_{0\ \text{E}}^{12}}{\text{AUC}_{0\ \text{BL}}^{12}} \tag{6}$$

where $\text{AUC}_{0\ \text{E}}^{12}$ is the area under the deactivation curve from 0 to 12 h and $\text{AUC}_{0\ \text{BL}}^{12}$ is the area under the trafficking curve from 0 to 12 h. Larger ABEC and smaller AUC_{SR} (which accounts for differing baselines) indicate greater net suppression.

The pharmacodynamic parameters for lymphocyte trafficking were determined by fitting cpm obtained with RU-40555 to eq 3, whereas pharmacodynamic parameters for lymphocyte deactivation were determined by simultaneously fitting cpm obtained with RU-40555 to eq 3 and cpm obtained without RU-40555 to eq 4. Prednisolone pharmacokinetic parameters were provided via eq 1. The ADAPT II.4 program (Biomedical Simulation Resource, Los Angeles, CA) was used for nonlinear regression fittings.

Statistical Aanalysis—The effects of gender on prednisolone CL, Vss, AUC_{SR}, ABEC, IC_{50T}, and IC_{50D} were assessed by an unpaired Student t-test, with statistical significance set at p < 0.05, using INSTAT (GraphPad Software, La Jolla, CA).

Results

Pharmacokinetics—Figure 2 shows mean plasma (± SD) concentrations versus time profiles of prednisolone in male and female rats. The disposition of prednisolone was biexponential, with a rapid initial phase followed by a slower elimination phase. Table 1 lists prednisolone pharmacokinetic parameters in the two groups. Mean (± SD) prednisolone clearance values were 3.22 ± 0.88 and 3.46

Table 1—Mean (± SD) Pharmacokinetic Parameters for Prednisolone in Male and Female Rats Following a Single iv Dose of 5 mg/kg (n = 4)

parameter	males		females	
	mean	SD	mean	SD
AUC (ng·h/mL)	1616	384	1526	436
MRT (h)	0.38	0.10	0.55	0.30
CL (L/h/kg)	3.22	0.88	3.46	0.96
Vd_{SS} (L/kg)	1.21	0.22	1.96	1.07
$t_{1/2}$ (h)	0.41	0.12	0.63	0.40

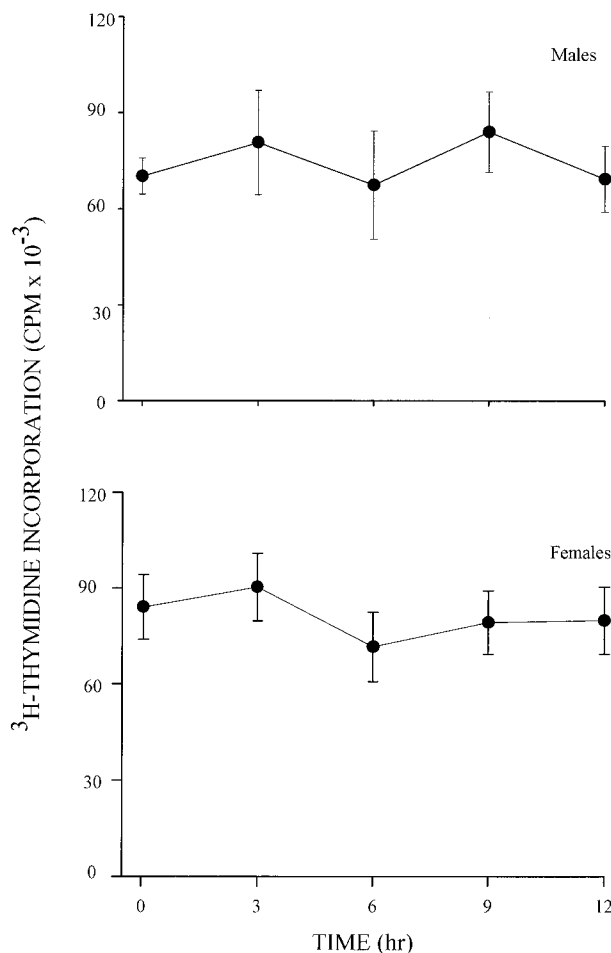**Figure 3**—[³H]Thymidine incorporation into whole blood T-cells as a function of time at baseline for male and females. Symbols represent mean (± SD) of data from four animals.

± 0.96 L/hr/kg in males and females, respectively. Mean prednisolone $t_{1/2}$ values were 0.41 ± 0.12 h in males and 0.63 ± 0.40 h in females.

Pharmacodynamics—*Baseline ³H-TDR Incorporation*—Figure 3 illustrates ³H-TDR incorporation into WBTC (relative cell counts) as a function of time at baseline in male and female rats. No statistical differences were observed between time points, suggesting that baseline levels were steady over 12 h.

In Vitro Interaction of RU-40555 with Prednisolone in WBTC Deactivation—Figure 4 shows the influence of RU-40555 on prednisolone inhibition of lymphocyte proliferation in vitro (deactivation). This agent completely antagonized the effects of the steroid at all concentrations investigated. Additionally, RU-40555 did not produce any changes in WBTC from control values.

Lymphocyte Trafficking—Figure 5 depicts mean ± SD relative WBTC counts and T-cell deactivation as a function
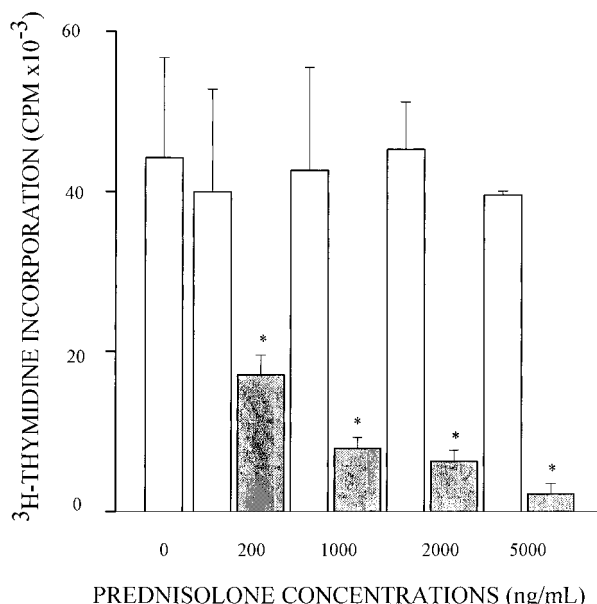
Figure 4—Joint effects of RU-40555 and prednisolone on whole blood T-cell deactivation in vitro. Key: (open bars) prednisolone + RU-40555 (250 mg/mL); (shaded bars) prednisolone alone; (*) $p < 0.05$ comparing single versus dual agents.
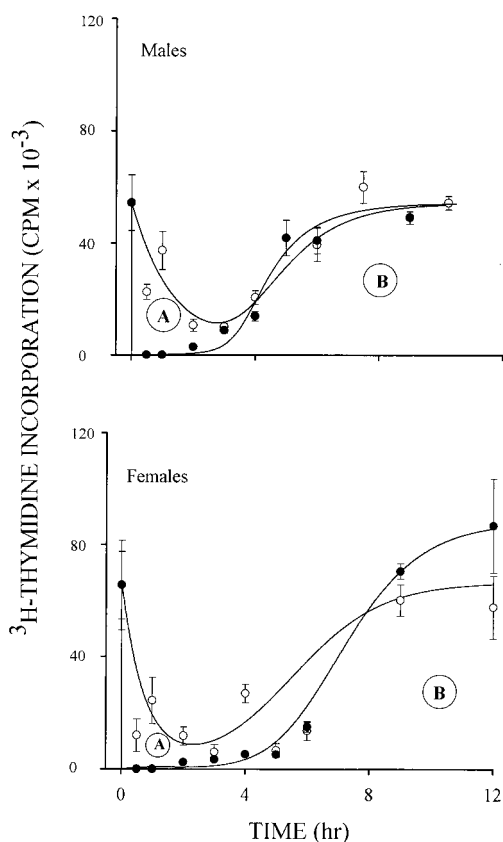

Figure 5—Mean ($\pm$ SD) [3H]thymidine incorporation into whole blood T-cells as a function of time in male and female rats after administration of prednisolone. Symbols are experimental data and lines represent fittings to the pharmacodynamic model. Key: (O) [3H]thymidine incorporation in the presence of RU-40555 (trafficking); (●) [3H]thymidine incorporation in absence of RU-40555 (deactivation). $AUC_{BL} = A + B$; $AUC_E = B$, and $AUC_{SR} = B/(A + B)$.

of time in male and female rats. After administration of prednisolone, the incorporation of ^{3}H-TDR into WBTC cultured with RU-40555 (relative WBTC counts) decreased slowly with time to reach a nadir at 3−5 h and returned

Table 2—Mean ($\pm$ SD)[a] Pharmacodynamic Parameters for Prednisolone Effects on Whole Blood T-Cell Trafficking and Deactivation in Male and Female Rats Following a Single iv Dose of 5 mg/kg

| | trafficking | | | | deactivation | | | |
| | males | | females | | males | | females | |
parameter	mean	SD	mean	SD	mean	SD	mean	SD
k_{in} (cpm/h) $\times 10^{-3}$	44.0[b]	4.2	119	38	NA[c]	NA	NA	NA
k_{out} (h^{-1})	0.73[b]	0.18	1.35	0.44	NA	NA	NA	NA
IC$_{50T}$ or IC$_{50D}$ (ng/mL)	0.14[b]	0.16	1.03	0.06	0.20	0.24	0.18	0.12
ABEC (cpm·h) $\times 10^{-3}$	NA	NA	NA	NA	130	80	232	208
AUC$_{SR}$	NA	NA	NA	NA	0.72	0.22	0.72	0.26

[a] All values represent the mean ($\pm$ SD) of four individual animals. [b] $p < 0.05$ as compared with the respective value in females. [c] NA, Not applicable.

to baseline levels by 8 h. Table 2 lists the pharmacodynamic parameters for trafficking and deactivation. Responses attained zero values and this complete inhibition allowed I_{max} to be set to 1. The mean ($\pm$ SD) prednisolone IC$_{50T}$ values for inhibition of WBTC trafficking was smaller in males compared with females (0.14 $\pm$ 0.16 versus 1.03 $\pm$ 0.06 ng/mL; $p < 0.05$). The net effect of prednisolone on lymphocyte trafficking (AUC$_{SR}$) could not be calculated because of the rebound effect observed in lymphocyte proliferation after the recovery of baseline in several animals (T-cell count rose above baseline levels).

Lymphocyte Deactivation—Following prednisolone administration, an immediate and complete inhibition of ^{3}H-TDR incorporation into lymphocytes cultured without RU-40555 was observed and baseline levels recovered slowly as prednisolone was cleared from blood (Figure 5). This response also showed complete inhibition, allowing I_{max} to be set to 1.0. The mean ($\pm$ SD) IC$_{50D}$ values for WBTC deactivation were similar in males and females (0.20 $\pm$ 0.24 vs 0.18 $\pm$ 0.12 ng/mL). A reduced ^{3}H-TDR incorporation into lymphocytes was still observed after prednisolone concentrations declined below the limit of quantitation (3 h post dose). There were no statistically significant differences between males and females with respect to the net deactivation of WBTC as shown by the mean AUC$_{SR}$ values (0.72 $\pm$ 0.22 in males and 0.72 $\pm$ 0.26 in females).

Discussion

To assess gender as a covariate in the optimization of glucocorticoid immunosuppressive therapy, we investigated WBTC trafficking and deactivation in male and female rats following a single dose of prednisolone. The pharmacokinetic parameters for prednisolone obtained in the present study were in the same range as those reported by Boudinot et al.[18] Although evidence exists supporting the role of sex steroid hormones in the regulation of transcortin (CBG), no statistical differences in prednisolone CL and steady-state volume of distribution (Vd_{ss}) between males and females were found (Table 1). These results may be explained by the relatively low dose used in this study (5 mg/kg). The binding of prednisolone to CBG is responsible for its dose-dependent disposition.[19] Unfortunately, we were unable to measure unbound prednisolone in this study.

Glucocorticoids exert some of their antiinflammatory actions by inhibiting the return of WBTC from the extravascular compartment to the blood compartment. In addition to such lymphocytopenia, these drugs deactivate T-cells to render them nonresponsive to peptide antigens. Glucocorticoids affect T-cell trafficking and deactivation via regulation of the secretion of cytokines, such as IL-2, and adhesion molecules.[22] Bloemena et al.[23] demonstrated that

decreased lymphocyte numbers in the peripheral blood are one of the determinant of the proliferative response of WBTC. Because proliferation of peripheral blood lymphocytes was measured in a standardized volume of blood (100 μL) at every time point, it was assumed that ^{3}H-TDR incorporation was determined by the proliferative capacity of individual lymphocytes as well as by the number of lymphocytes present in the peripheral blood. Thus the incorporation of ^{3}H-TDR into whole blood cultures was used to simultaneously determine the effects of prednisolone on WBTC trafficking and their intrinsic activation.

Relative WBTC number was determined by using the glucocorticoid receptor antagonist RU-40555 added to ex vivo cultures of whole blood from dosed animals.[24] This agent antagonizes prednisolone deactivation of WBTC in vitro (Figure 4) in the same concentration range as that obtained with a 5-mg/kg dose (Figure 2). The concentration of RU-40555 utilized did not affect WBTC (Figure 4). Therefore, the incorporation of ^{3}H-TDR into whole blood cultures measured in the presence of RU-40555 could be taken as the relative number of WBTC present at each time point. In normal humans, lymphocyte trafficking between blood and extravascular compartments parallels the circadian variation of endogenous cortisol (the number of lymphocytes shows a clear rhythmicity with high values at night and low values during the day).[25] In this study, we utilized adrenalectomized animals to suppress the effect of the latter because the adrenal gland is the main source of endogenous glucocorticoids. This objective was achieved because the incorporation of ^{3}H-TDR into whole blood cultures at baseline was stable over 12 h (Figure 3). Whole blood lymphocytes decreased after administration of prednisolone. This time profile (Figure 5) was similar to that obtained in humans. The maximum response was delayed with respect to prednisolone concentrations, showing that our method describes well the movement of cells between the blood and extravascular compartments. An innovative PK/PD model that incorporates features of indirect effects of prednisolone on cell trafficking as well as direct inhibition of ex vivo T-cell activation was used.[16] The model described well the experimental data, and pharmacodynamic parameters were obtained with relatively good precision (5−20% CV). Prednisolone had a significantly smaller IC_{50T} in males compared with females. This result show that male rats may be more sensitive than females to inhibition of WBTC trafficking by prednisolone. These observations may be due to gender differences in estrogen levels. Indeed, estrogen receptors are found on lymphoid cells.[26] Estradiol inhibition of polymorphonuclear leukocyte chemotaxis is mediated by estrogen receptors.[27] Also, estradiol down-regulates the expression of P-selectins, molecules involved in the regulation of cell trafficking.[28]

Following prednisolone administration, an immediate and complete inhibition of ^{3}H-TDR incorporation into lymphocytes was observed in whole blood without RU-40555. Baseline values were recovered slowly as prednisolone was cleared from blood. The diminished responsiveness of WBTC to stimulation with Con-A (intrinsic deactivation) appeared to be a direct effect of prednisolone because the degree of inhibition of ^{3}H-TDR incorporation paralleled that of prednisolone concentrations. This result required very small steroid concentrations. Indeed, considerable inhibition remained when the drug fell below the limit of quantitation. The sensitivity of WBTC to prednisolone deactivation was not affected by gender as no statistical differences were observed in the mean IC_{50D} and AUC_{SR} (net suppression) values between males and females. Ferron and Jusko[29] also recently found no gender differences in in vitro WBTC effects of prednisolone in adrenalectomized rats and in humans.

In conclusion, by using a glucocorticoid receptor antagonist, we were able to monitor simultaneously two major components of glucocorticoid immunosuppression (trafficking and deactivation). The use of RU-40555 allowed separation of these phenomena, permitting estimation of IC_{50} values for prednisolone effects on each component. Greater immunosuppression was achieved overall in male rats compared with females following a 5-mg/kg single dose of prednisolone. However, no gender differences were observed in either the exposure to the drug or WBTC deactivation.

Abbreviations

^{3}H-TDR	[^{3}H]thymidine
WBTC	whole blood T-cell
Con-A	concanavalin-A
IC_{50T}	concentration producing 50% inhibition of the zero-order rate constant (k_{in}) for return of blood T-cells from the extravascular compartment to the blood compartment
IC_{50D}	concentration producing 50% inhibition of whole blood T-cell activation
I_{max}	maximum inhibition of the zero-order rate constant (k_{in}) for return of blood T-cells from the extravascular compartment to the blood compartment or maximum inhibition of whole blood T-cell activation.
ABEC	area between baseline and effect curves
AUC_{SR}	ratio of the area between the effect curve and the area at baseline
cpm	counts per minute
k_{in}	zero-order rate for return of lymphocytes from the extravascular compartment to the blood compartment
k_{out}	first-order rate constant for exit of lymphocytes from the blood compartment
CL	Clearance
Vd_{ss}	apparent volume of distribution at steady state
$t_{1/2}$	terminal half-life
MRT	mean residence time
AUC	area under the plasma concentration−time curve
AUMC	area under the first moment curve

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