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Immunodynamics of Methylprednisolone Induced T-cell Trafficking and Deactivation Using Whole Blood Lymphocyte Proliferation Techniques in the Rat

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ABSTRACT: Glucocorticoids have diverse effects on various components of the immune system and assessment of such activities *in vivo* often involves complex techniques and numerous animals. We developed a whole blood technique for determining proliferation rate of lymphocytes in minute amounts of rat blood (5 μL as opposed to a whole rat spleen) (Fasanmade AA, Jusko WJ. *J Immunol Methods* 1995; **184**: 163–167). This method was used in assessment of *in vivo* T-cell deactivation by methylprednisolone (MP). The blockade of this process by the anti-glucocorticoid, RU 40555, also allows measurement of T-lymphocyte trafficking between vascular and extravascular pools. Blood samples were taken over several hours after iv MP administration to adrenalectomized rats, MP concentrations and lympho-proliferative activities were determined *ex vivo* after mitogen activation with and without blocking MP with RU 40555. MP disposition was mono-exponential with a $t_{1/2}$ of 34 min. The pharmacodynamics (PD) of T-cell trafficking was modeled with a physiological indirect model to generate the IC_{50} (0.4 ng/mL) for the inhibitory action of MP on return of T-cells to blood as well as cell trafficking rate constants. The overall suppression of blood T-cells was modeled with an equation which accounts directly for inhibition of the proliferation activity of available blood T-cells with an DC_{50} of 0.37 ng/mL. MP produced an initial influx of T-cells to blood within 1 h of infusion, a later marked T-cell depletion with a nadir at 4 h, and return to baseline by 9 h. Lymphocyte deactivation occurred within minutes of MP infusion and returned to baseline in 9 h. MP action was prolonged owing to the low IC_{50}. This approach for assessing dual features of corticosteroid effects on T-cell trafficking and deactivation allows quantitative PK/PD modeling in small animals such as the rat. Copyright © 1999 John Wiley & Sons, Ltd.

Key words: methylprednisolone; corticosteroids; immunosuppression; glucocorticoid antagonist

Introduction

Glucocorticoids modulate the mammalian immune system by suppressing production of endogenous corticosteroids [1] and several cytokines [2]. These cytokines affect the functions or proliferation capacities of cells of the immune systems. The presence of glucocorticoids in blood causes migration of subsets of lymphocytes to and from the central vascular compartment. Glucocorticoids reduce the functional capacities of lymphocytes [3] and can degenerate organs responsible for their maturation. These compounds can down-regulate their own receptor sites by suppressing receptor regeneration or production.

Several methods are used in the *ex vivo* assessment of the potency of an *in vivo* administered immunosuppressant. Lymphocyte migration or trafficking can be studied by flow-cell cytometry. This method employs small volumes of blood and has been used for assessing effects of glucocorticoids on lymphocyte trafficking to and from the central blood compartment in the rat [4]. This method allows an accurate measurement of the population density of lymphocytes at various time intervals after steroid dosing. However, the technique does not account for the total effects of glucocorticoids on lymphocytes as it does not measure the intrinsic suppressive activity on lymphocyte proliferation.

The suppressive effect of glucocorticoids on lymphocyte proliferation is usually determined by separation of lymphocytes from large volumes (5 mL or more) of blood by the FICA Pique method and determining the proliferative rate of fixed number of lymphocytes [5,6]. While this method is very useful in humans and larger animals, it is not applicable in small laboratory animals such as the rat because of the amount of blood required. Instead, splenocytes from sacrificed animals are used. Even when lymphocyte isolation is used in larger species, it fails to account for lymphocyte migration since a specific number of isolated cells are employed in such studies. Whole blood has been previously employed to assay lymphocyte proliferation rates [7,8],

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and the procedure was optimized for studies in the rat [9]. This procedure employed volumes of blood as low as 5 μL allowing measurement of the intrinsic proliferative activities of the T-cells at many time points after steroid dosing.

In the present study, the whole blood lymphocyte proliferation technique was used to determine the overall effects of methylprednisolone on the functional activities of T-cells of treated rats at various time intervals after an intravenous dose of the drug. Furthermore, the technique was extended to determine the movement of lymphocytes to and from the central vascular compartment by blocking the effect of methylprednisolone with an anti-glucocorticoid hormone, RU 40555. Thus, the contribution of methylprednisolone to the trafficking and functional suppression of lymphocyte was determined simultaneously in the same animals. Pharmacokinetic–pharmacodynamic (PK/PD) models are proposed for the description of the observed responses.

Materials and Methods

Animals and Dosing

All animals used in these studies were treated in adherence to the principles of Laboratory Animal Care [10]. Male Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN, USA) weighing 280–380 g were used. The rats were adrenalectomized and were acclimatized in a 12 h light and 12 h dark cycle for 2 weeks before being used in this study. The animals had free access to rat chow (Agway RMH 1000) and normal saline (NaCl, 0.9%) prior to the experiments. One day prior to study, animals were anesthetized with a mixture of ketamine and xylazine (60 and 2.5 mg/kg) and silastic cannulas were surgically implanted in the right external jugular vein. Cannulas were kept patent with saline containing 50 U/mL heparin without preservatives. The rats were randomly assigned to receive either 5 mg/kg of MP as methylprednisolone sodium succinate (Sigma, St. Louis, MO, USA) or normal saline. At approximately 09:00 h, drug or saline were administered through the jugular vein cannula. Blood samples were taken for lymphocyte proliferation and MP assays at time 0 (just before MP administration), 5, 15, 30, 45, 60, 120, 180, 240, 360 and 540 min after MP dosing. After each blood sample, the same volume of saline was re-infused through the cannula.

Quantitation of Methylprednisolone

Methylprednisolone was determined in blood using an earlier method [11]. Briefly, 0.2 mL of blood sample was deproteinated with 2 mL of acetonitrile and the supernatant extracted into 15 mL of methylene chloride and 1.5 mL of water, the organic layer was dried at 30°C under a stream of nitrogen. The residue was reconstituted in the mobile phase and injected onto a high performance liquid chromatographic (HPLC) column. The HPLC system consisted of a Waters 210 pump (Waters Inc., Milford, MA, USA), an octadecyl silica column (i.d. 4.6×150 mm) and a Waters 486 UV detector. The limit of quantitation (LOQ) was 5 ng/mL.

Proliferation Assay

Tissue culture medium, RPMI 1640 (Gibco, Grand lsland, NY, USA) was supplemented with 1 mM glutamine, 20 mM Hepes buffer, 100 U/mL streptomycin, 0.25 mM 2-mercaptoethanol and 7.5% heat inactivated fetal calf serum. A 35 μL aliquot of whole blood was diluted to 700 μL with this medium within 1 min of sampling. For each time point, 100 μL of the diluted blood was dispensed into each of six wells of a 96-well round bottom polystyrene multi-well plate (Corning, NY, USA). Mitogen, concanavalin A (Con A) from ICN Biochemicals (Cleveland, OH), was added in 10 μL of culture medium to give a final concentration of 10 μg/mL in the well. To three of the wells, 50 μL aliquots were added of a solution of RU 40555 (Roussel Uclaf, Paris, France) in RPMI 1640 such that the final concentration in the well was usually 100 ng/mL. Media was added to each well to give a total tissue culture volume of 250 μL. Blood was diluted within 1 min of collection and incubated within 30 min of sampling at 37°C under a humidified atmosphere of 7% CO_2 for 72 h. Then 1 μCi of ^{3}H-thymidine per well was added and incubated for an additional 18 h. Cells were harvested with an automatic harvester (Skatron Instruments, Sterling, VA, USA). The proliferation rate (cellular incorporated H-TdR) was assessed in counts per minute (CPM) by radiometry using a Packard 1900 CA Tri-Carb liquid scintillation counter (Downers Grove, IL, USA).

In a preliminary experiment, 5 mL of blood from a rat was depleted of leukocytes by centrifuging at $1500 \times g$ for 15 min. The plasma layer was removed but not discarded, the buffy coat layer was carefully removed with as few red cells as possible and discarded, and then the plasma was reconstituted with the red cells.

Rat mononuclear cells from another 5 mL of blood were isolated using Fico/Lite as described previously [9]. Briefly, the cells at the interphase were removed and washed three times with RPMI. The cell pellet was re-suspended in supplemented RPMI, and a total count was performed using an haemacytometer. The viability of the lymphocytes was $>95\%$ when determined by the 0.2% trypan blue dye exclusion technique. The isolated cells were returned to the leukocyte depleted blood to

give the required number of cells/μL to study the variation in proliferative rate with cell number.

Data Analysis

Proliferation Rate. The mean basal ^{3}H incorporation (CPM_b) in wells of blood samples taken before MP administration without the addition of mitogen was determined. The proliferation rate at time zero (CPM_0) was determined by subtracting CPM_b from the mean of CPM determined from blood sample taken just before MP administration but activated *ex vivo* with mitogen. The proliferation rate of T-cells in the blood samples obtained at time t after MP administration (CPM_t), was determined in a similar manner to CPM_0. The net effect of MP on the T-cells in the blood at time t was determined as $CPM_0 - CPM_t$.

Estimation of T-cell Trafficking and Deactivation Due to MP in the Blood. The total uptake of ^{3}H-thymidine at supra-optimal stimulation of the T-cells depends on the number of these cells present in the sample and is inversely related to the suppressive effect of MP. When the anti-proliferative effect of MP is totally blocked, and other conditions were kept constant, then ^{3}H-thymidine uptake at supra-optimal stimulation depends solely on the number of cells present in the well. Thus, variations in *ex vivo* optimal stimulation of T-cells after MP action has been blocked with RU 40555 is proportional to trafficking of T-cells between vascular and extravascular sites. The counterpart values of CPM_b, CPM_0 and CPM_t determined in the presence of large excess of RU 40555 in the wells are: CPM_{ru_b}, CPM_{ru_0} and CPM_{ru_t}. The amount of RU 40555 required for these experiments was determined in preliminary studies. T-cell trafficking was defined as the net percentage of the original number of intravascular T-cells that migrated out of the central vascular system. Thus, the trafficking effect, E_{tr}, is given by:

$$E_{tr} = [(CPM_{ru_0} - CPM_{ru_t})/CPM_{ru_0}] \cdot 100. \quad (1)$$

The value used in the modeling of the trafficking pharmacodynamics is [Tcell] given by:

$$[\text{Tcell}] = 100 - E_{tr} \text{ or } 100 \cdot CPM_{ru_t}/CPM_{ru_0}, \quad (2)$$

which is the proliferative rate of whole blood T-cells in the presence of RU 40555 and represents the blood population of T-cells. The profile of the total effect of MP obtained above as CPM_t is represented by [LYM], and reflects the proliferative activity of T-cells when the RU 40555 was not added.

Pharmacokinetic and Pharmacodynamic Models

Pharmacokinetics. The pharmacokinetics of methylprednisolone were modeled using the WinNonlin software (PharSight Corp, Apex, NC, USA). The MP blood concentration, C_b was fitted to:

$$C_b = C_b^0 \, e^{-k_{el}t}, \quad (3)$$

where C_b^0 was the initial concentration at a time $t = 0$ just after the iv dose of MP was given and k_{el} is the elimination rate constant.

Models for Cell Trafficking Dynamics. The pharmacodynamic models are structured with blood and extravascular compartments accounting for cell trafficking (Figure 1). The rate of change in basal number of T-cells in the vascular compartment can be represented by:

$$d[\text{Tcell}]/dt = k_{return} - k_{out}[\text{Tcell}], \quad (4)$$

where k_{return} is the zero-order rate constant describing T-cell migrating into blood and k_{out} is the first-order rate constant for T-cell outflow from blood. Alteration in the leukocyte content in blood after administration of corticosteroids have been shown to be due to the inhibition of these cells from entering blood from extravascular sites [12]. Thus, indirect response model I with inhibiton of k_{return} was used [13,14] as:

$$d[\text{Tcell}]/dt = k_{return} I_t - k_{out}[\text{Tcell}], \quad (5)$$

where the inhibition function is:

$$I_t = 1 - (C_b/(C_b + IC_{50})), \quad (6)$$

with IC_{50} the MP concentration at which the k_{return} pathway is inhibited by 50%. Because of the biphasic nature of T-cell trafficking under the influence of MP, a hypothetical extravascular compartment which is a subset of the global extravascular compartment was assumed to be responsible for an initial upsurge in blood population of T-cells [4]. This is depicted as compartment S in Figure 1. This was modeled with a stimulatory function adding T-cells to the blood with $k_{release}$ that is influenced by MP; thus, the final model for cell trafficking is:

$$d[\text{Tcell}]/dt = k_{return} I_t + E_t k_{release} - k_{out}[\text{Tcell}], \quad (7)$$

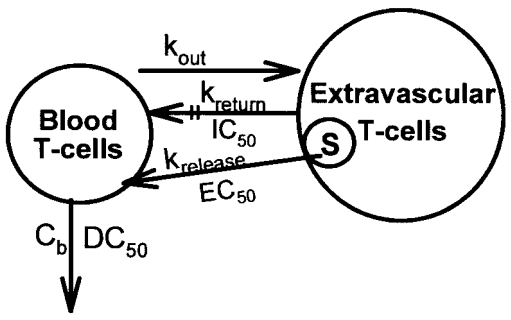

Figure 1. T-cell trafficking model. The model depicts the movement of lymphocytes between vascular and extravascular sites. Symbols are: k_{out}, the exit rate constant of cells from blood; k_{return}, the return rate constant of cells to blood; $k_{release}$, the rate constant of steroid-induced release of cells into the circulation; IC_{50}, the MP concentration inhibiting 50% of the cell return pathway; EC_{50}, the MP concentration inducing 50% of the release pathway; S, a sub-compartment where cell release is controlled by MP

where E_t is given by:

$$E = C_b/(C_b + EC_{50}), \quad (8)$$

with EC_{50} the concentration of MP that causes a 50% increase in whole blood T-cells.

Model for T-cell Deactivation Dynamics. In addition to its effects on cell trafficking, glucocorticoids suppress T-cell responsiveness. This was measured by the ability of T-cells to proliferate in response to activation by mitogen at the supra-optimum stimulatory condition.Thus, the rate of change of T-cell proliferation activity can be expressed as:

$$d[LYM]/dt = [Tcell]_t(1 - C_b/(C_b + DC_{50})) - kd_{out}[LYM], \quad (9)$$

where DC_{50} is the MP concentration causing 50% deactivation of proliferation compared to the supra-optimum proliferation rate and [LYM] is the pro liferative activity of blood T-cells when RU 40555 was not added to the test wells. At time t, after MP administration, the observed proliferation rate, $[Tcell]_t$, is proportional to the number of T-cells in the blood. The kd_{out} process can be viewed as a first-order physiological 'wearing out' of corticosteroid effects on the mitogen generated proliferation enhancing process, that is, the rate at which cells resume optimum production of cytokines such as IL-2 after a blockade by MP but under the influence of mitogens. Thus, in modeling the deactivation by MP, the above equation is solved with input of $[Tcell]_t$ values from Equation (7) with the DC_{50} characteristic of *in vivo* deactivation by MP *in vivo*.

Results

Blockade of the Anti-proliferative Effects of MP by RU 40555

When mononuclear cells from rat peripheral blood were stimulated with Con A at supra-optimum conditions using the whole blood assay technique, uptake of ^{3}H-thymidine increases with numbers of mononuclear cells (Figure 2(a)). The increase varies linearly and rapidly when the number of mononuclear cells were between 5000 and 50000 in the diluted blood in wells of a 96-well plate; outside this range the increase in ^{3}H-thymidine uptake was not linear. At mononuclear cell numbers within the linear range, ^{3}H-thymidine uptake as measured in CPM reflects the number of T-cells present in the test wells when all conditions were kept constant and using adrenalectomized rats. The linear range was maintained in subsequent experiments in this study.

RU 40555, a compound structurally related to the glucocorticoid hormones is known to block several

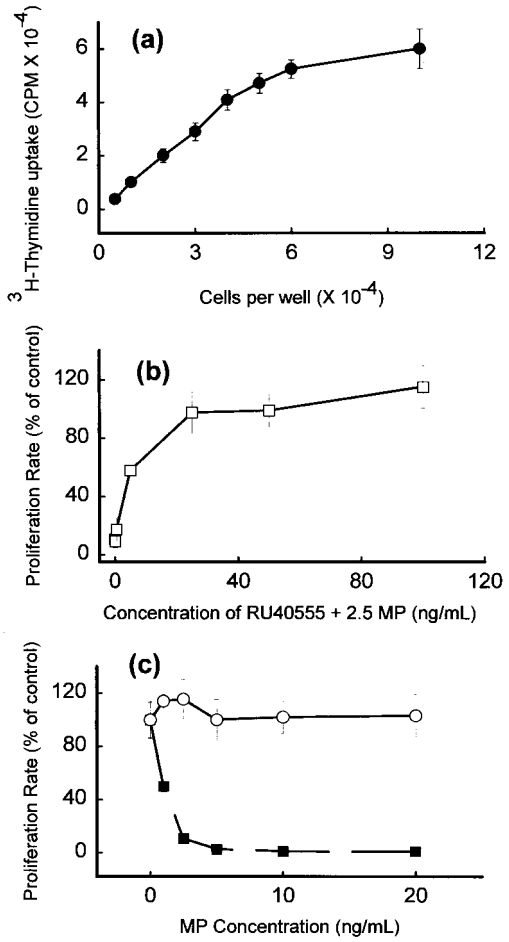

Figure 2. Effects of MP and RU 40555 on lymphocyte proliferation rate at supra-optimal mitogenic induction. (a) Relationship between ^{3}H-thymidine uptake measured by scintillation CPM and number of mononuclear cells per well. (b) Suppression of T-cell proliferation by methylprednisolone (MP) and blockade of anti-proliferative action of MP in the whole blood lymphocyte proliferation assay by RU 40555. All wells had 2.5 ng/mL of MP plus the indicated concentrations of RU 40555. The MP alone suppressed lymphocyte proliferation to 10% of untreated supra-optimal activated controls. (c) Blockade of the anti-proliferative effect of MP using RU 40555: The broken line represents proliferation rate in presence of the stated concentrations of MP only. The open symbols represent proliferation rate in presence of the stated MP concentrations in combination with 100 ng/mL RU 40555

of the actions of glucocorticoid hormones [15]. Its specific activity on the anti-proliferative action of methylprednisolone was examined. Figure 2(b) shows that when whole blood samples were stimulated with a supra-optimum concentration of Con A, 2.5 ng/mL of MP concentration decreased the proliferation rate of the T-cells to 10%. However, the addition of increasing concentrations of RU 40555 (to the MP-suppressed cells) brought the proliferation rate back to the original levels. The reversal of the activity of MP is RU 40555 concentration-dependent up to 25 ng/mL; above this concentration, the anti-proliferative effects of MP were no longer observable. Thus, for subsequent experiments where we wanted to remove the influence of

the direct presence of MP on T-cell proliferation, 100 ng/mL of RU 40555 was employed, a concentration well above that required to suppress the anti-proliferative effects of MP but with no observable adverse effects on the T-cells as shown in Figure 2(c). MP is shown to inhibit proliferation of the T-cells down to less than 1% of control cultures in a concentration dependent manner, but addition of 100 ng/mL of RU 40555 completely protected these cells from the anti-proliferative effects of MP. The prior presence of MP in the cell culture did not prevent RU 40555 from expressing its anti-MP action when RU was applied prior to, or simultaneously with, mitogen. Under these conditions the observed proliferative capacity is directly related to the number of viable T-cells present in the test-wells.

Pharmacokinetics of MP. A typical profile of MP concentration in blood of a rat is shown in Figure 3. MP disposition follows first-order kinetics with $t_{1/2}$ of about 30 min. These kinetics are similar to those reported in earlier studies for plasma or serum. Table 1 lists the PK parameters of MP.

Pharmacodynamics. Two effects of MP were studied in the current work: the trafficking of T-cells between the central and extravascular compartments and the deactivation of T-cells. The overall effects of a dose of MP on blood lymphocyte proliferation rate determined *ex vivo* on serial blood samples taken after an iv dose of MP is depicted in Figure 3. This profile is a result of the composite effects of MP on trafficking and proliferation of lymphocytes. *In vivo*, MP is known to influence the rate of movement of T-cells in and out of the vascular system [12]. Blood samples taken after MP administration contain fractions of T-cells representative of the current vascular content of these cells. These samples also contain concentrations of MP that will continue to suppress lymphocyte proliferation. Previous studies have demonstrated that even after glucocorticoid levels have decreased to undetectable amounts in the plasma, their anti-proliferative effects still continued to be manifested for longer periods of time [16]. Thus, we expect that the activation observed after *ex vivo* mitogen stimulation of T-cells in the whole blood sample will be affected by both the number of these cells and current MP concentration in the blood (Figure 3).

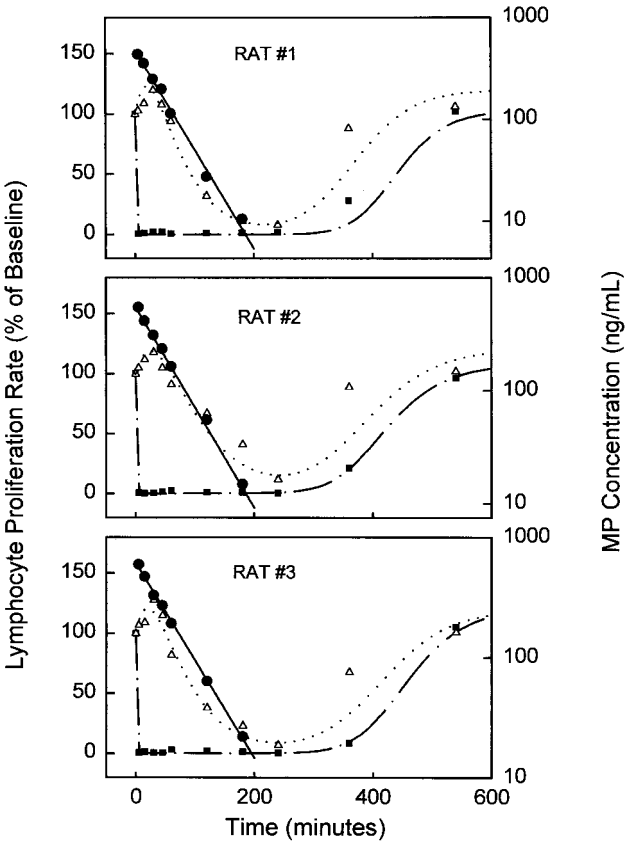

Figure 3. Blood profiles for MP and T-cells in rats after an iv dose of 5 mg/mL MP. The symbols are actual experimental values from a representative animal while the lines represent the model predicted values. Circles are blood concentrations of MP; triangles are T-cell trafficking profiles; and squares are total suppressive effects of MP on T-cells

Table 1. Pharmacokinetic and pharmacodynamic parameters for MP (mean (S.D.))

Parameters	
Kinetics ($n = 3$)	
AUC (μg · hr/mL)	26.9 (1.6)
$t_{1/2}$ (min)	33.9 (1.7)
C_{max} (ng/mL)	549 (48)
Cl (mL/min/kg)	186 (11)
V_{ss} (L/kg)	9.09 (0.80)
Dynamics ($n = 3$)	
IC_{50} (ng/mL) (trafficking)	0.401 (0.174)
k_{return} (% cells/min)	3.74 (1.12)
$k_{release}$ (% cells/min)	42.9 (16.0)
k_{out} (min^{-1})	0.0312 (0.0094)
EC_{50} (μg/mL) (initial release)	3.73 (0.81)
DC_{50} (ng/mL) (deactivation)	0.370 (0.050)
kd_{out} (min^{-1})	1.090 (0.092)

In order to delineate the different profiles we blocked the anti-proliferative effects of MP with RU 40555 such that the observed response only reflects the number of T-cells in the blood samples. The results in Figure 3 show that after MP administration, the number of T-cells increased starting from 5 min to a maximum within 1 h after MP administration. The increase in cell numbers was around 20% over the pre-dose baseline and is statistically significantly different from those of control animals treated with saline. After the initial rise, the population of T-cells in blood declined rapidly to a nadir of 10% of baseline value of about 4 h after the MP dose. A gradual recovery to the baseline value occurs in about 9 h. The data were fitted to Equations (3) and (7), and typical fitted curves are shown in Figure 3. The parameter values are listed in Table 1.

The initial rise in the number of cells requires a high blood concentration of MP as predicted by the large value of EC_{50} of 3.7 μg/mL which controls the rate of release of T-cells from extravascular sources. This is in line with the fact that this rise is observed only at times in the pharmacokinetic profile when C_b is high.

The total effect of MP on the deactivation of T-cells *in vivo* in the blood compartment was modeled using Equations (3), (7) and (9) reflecting the fact that the *ex vivo* measured suppression of proliferation is performed only on the remaining cells after trafficking. The parameter (Table 1) generated by this equation is applicable to the anti-proliferative effect of MP *in vivo* and takes into consideration trafficking phenomenon since the current number of vascular T-cells were used in the model. Thus the parameter, DC_{50}, expresses the intrinsic activity of MP on the deactivation of blood T-cells. The equation therefore is able to define the intrinsic anti-proliferative property of MP in an *in vivo* drug assessment. While the value of 0.37 ng/mL is below the LOQ of MP of 5 ng/mL, the steroid commonly exhibits continued elimination at the observed terminal half-life.

Discussion

Whole blood concentrations of MP were measured in this study because it is more directly relevant to the effects of MP in the *ex vivo* proliferation experiments which is also based on whole blood. Since we employed only 5 μL of blood per well in the 96-well plates in a final culture volume of 250 μL, there is a dilution factor of 1:50. It is known that after the initial exposure of mononuclear cells to corticosteroids *in vivo*, anti-proliferative effects are maintained even when the cells were isolated and washed before testing for immunosuppressive activity *ex vivo* without further addition of the test drug; this has been a common practice [16]. This could be due to the receptors in the cells having been tightly bound to and internalized the drug and thus were not removable during washing or the fact that the cellular machinery for the response cascades had begun before isolation of cells from the *in vivo* drug-rich environment. Therefore, for the current technique, where there is no need for prior isolation of the cells from blood, the 50-fold dilution made the final drug carried over too low to be the sole factor controlling the proliferation rate of the cells, rather, a major contributory factor is the original *in vivo* exposure of cells to MP.

The assessment of immunosuppressants *in vivo* is known to be influenced by various endogenous components of plasma that may interfere with immunosuppressive activities [17]. Corticosterone is especially of concern in this type of study in the rat, not only because of its potency and abundance, but also because of its fluctuating plasma concentrations under the influence of various stimuli [18]. Use of adrenalectomized rats minimizes these concerns and allows a more controlled experimental condition. Thus, our choice of such rats for the current study allows us to attribute responses solely to MP.

The immunosuppressive activities of corticosteroids are known to be expressed through glucocorticoid receptor binding leading to their effects at the transcription level and gene expression. On the other hand, the stimulation of T-cells by mitogens such as Con A is through a series of signal transduction steps involving the mitogen activated protein kinases (MAPK), these kinases phosphorylate endogenous proteins which eventually lead to induction of their mRNA and expression of genes. Thus, several interleukins, including IL-2 are produced in this process by T-cells. IL-2 is required in the rapid proliferation process of the T-cells. At supra-optimum mitogen activated proliferation conditions which we employed in this study, Con A treatment should lead to optimum production of IL-2 which is kept at a steady-state during the assay period by its physiological rate of degradation. The control of production of IL-2 and its receptors on T-cells by glucocorticoids is therefore an indirect control of lymphocyte proliferation rate. This process serves as the basis for the inhibitory mode of one of the physiologic indirect models to link the pharmacokinetics of MP to its pharmacodynamic effects.

The trafficking pattern observed here is similar to what has been reported earlier using fluorescence activated flow cytometry sorting (FACS) [4]. MP, like other reported corticosteroids, has mixed trafficking effects on lymphocytes. The initial rise is most probably a result of MP effect on certain subsets of lymphocytes. It has been shown by several groups that corticosteroids produce an influx of, for example, natural killer (NK) cells ($CD3-$, $CD4-$, $CD8+$) into the blood [19]. Similarly, exercise which causes a rise in hydrocortisone has similar effects [20]. This phenomenon has been quantitated in humans using prednisolone [21] and gave results that are similar to part of our results. Migration from the blood of other cells such as basophils [22] and helper-T lymphocytes [12] is also caused by these steroids. Thus, the overall effect observed when using a method that assesses lymphocyte migration as a composite is the net effect on the various subsets of T-cells. Similar results have been reported using other general surrogates to measure trafficking of T-cells in the blood after exposure to immunosuppressive agents [22]. The anti-proliferation effect of glucocorticoids is, however, more uniform for the T-cells and has been used extensively in the assessment of immunosuppressive activities of many agents.

The current technique, using minute amounts of whole blood and blocking glucocorticoid activities with a specific antagonist, provides efficient assessment of the activities of these drugs. It was employed to assess gender differences in prednisolone effects in rats [23]. The ability of the method to delineate between lymphocyte suppression and migration in a single experiment with one technique makes the method of additional mechanistic value. The simplicity and the large difference in cost between this method and other methods of *in vivo* assessment of drugs affecting lymphocyte deactivation and other immunosuppressive parameters is noteworthy. For example, a complete *in vivo* pharmacokinetic, lymphocyte deactivation, and trafficking pharmacodynamic profile are obtained from a single live rat which remains healthy throughout and after the experiments (replicate animals are needed only for statistical purposes). The savings is obvious when compared to experiments which required 33 animals to be sacrificed for serial measurements where splenocytes are collected [16]. This method does not produce cell trafficking information, although this could be readily obtained by flow cytometry. Thus, the current method presents significant improvements over previously described methods of assessing corticosteroid pharmacodynamics in the rat.

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