Published in final edited form as:

Ther Drug Monit. 2014 August ; 36(4): 448–455. doi:10.1097/FTD.0000000000000045.

Pharmacokinetics of Total and Unbound Prednisone and Prednisolone in Stable Kidney Transplant Recipients with Diabetes Mellitus

Ileana A. Ionita^{1,2}, Ken Ogasawara¹, Reginald Y. Gohh³, and Fatemeh Akhlaghi^{1,*}

¹Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI, USA

²Department of Pharmacokinetics Dynamics and Metabolism, Pfizer Inc., Groton, CT, USA

³Division of Organ Transplantation, Alpert Medical School of Brown University, Providence, RI, USA

Abstract

Background and Objective—The corticosteroid prednisone is an important component of post transplantation immunosuppressive therapy. Pharmacokinetic parameters of prednisone or its pharmacologically active metabolite, prednisolone, are not well characterized in transplant recipients. The objective of the present study was to compare the pharmacokinetics of total and unbound prednisone and prednisolone in diabetic and nondiabetic stable kidney transplant recipients and to evaluate the factors influencing plasma protein binding of prednisolone.

Study Design—Prednisone and prednisolone concentration-time profiles were obtained in 20 diabetic and 18 nondiabetic stable kidney transplant recipients receiving an oral dose of 5-10 mg prednisone per day. In addition to drug and metabolite exposures, factors influencing prednisolone protein binding were evaluated using a nonlinear mixed effects modeling approach. This model takes into account binding of prednisolone and cortisol to Corticosteroid Binding Globulin (CBG) in a saturable fashion and binding of prednisolone to albumin in a non-saturable fashion. Finally, we have investigated the influence of several covariates including diabetes, glucose concentration, hemoglobin A_{1c}, creatinine clearance, body mass index, gender, age and time post transplantation on the affinity constant (K) between corticosteroids and their binding proteins.

Results—In diabetic patients, the values of dose-normalized area under the concentration-time curves were 27% and 23% higher for total and unbound prednisolone, respectively. Moreover, the ratio of total prednisolone to prednisone concentrations (active /inactive forms) was higher in diabetic subjects (P <0.001). Modeling protein binding results revealed that the affinity constant of CBG-prednisolone (K_{CBG,PL}) was related to patient's gender and diabetes status.

Conclusions—Higher prednisolone exposure could potentially lead to the increased risk of corticosteroid related complications in diabetic kidney transplant recipients.

Name and address for correspondence: Fatemeh Akhlaghi, Pharm.D., Ph.D., Clinical Pharmacokinetics Research Laboratory, Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, 7 Greenhouse Rd., Kingston, RI 02881, USA, Telephone: (401) 874-9205, Fax: (401) 874-5787, fatemeh@uri.edu.

Keywords

11 β -HSD1; corticosteroid binding globulin; diabetes; pharmacokinetics; prednisolone; protein binding; unbound

Introduction

Prednisone (predominantly administered in the USA) and its active metabolite prednisolone (administered in Europe), are a component of immunosuppressive regimens for kidney transplant recipients [1], although attempts have been made to reduce or eliminate their use following transplantation [2]. Doses up to 10 mg/day prednisone are typically administered in addition to a calcineurin inhibitor, cyclosporine or tacrolimus, and an anti-proliferative agent, mycophenolic acid (MPA).

Earlier studies performed in our laboratory have shown that the concentrations of some of the cyclosporine metabolites are reduced in diabetic when compared with nondiabetic stable kidney transplant recipients [3]. Moreover, the metabolite ratio of MPA-glucuronide and Acyl-MPA-glucuronide was higher [4, 5] and the catalytic activity of inosine monophosphate dehydrogenase (molecular target of mycophenolic acid) was reduced in diabetic patients [4].

Approximately 30% of all kidney transplant recipients in the United States have diabetes mellitus before transplantation and an additional 10% develop new-onset diabetes after transplantation (NODAT) within one year after the transplant operation [6]. Previous studies have found that diabetic transplant recipients are hospitalized more often due to infections and have higher hospital mortality [7]. Because immunosuppressive activity of prednisolone, measured as the percentage inhibition of the mixed lymphocyte reaction, was lower in patients with lower exposure of unbound prednisolone [8], higher concentrations of glucocorticoids in diabetic subjects could potentially contribute to the increased risk for infections due to over-immunosuppression.

Prednisolone suppresses the endogenous production of cortisol by inhibiting the hypothalamic-pituitary-adrenal axis [9]. Kidney transplant recipients treated with prednisolone and azathioprine or cyclosporine had a higher risk of rejection when their morning cortisol concentrations were greater than 4 μ g/L. Cortisol concentrations below 1 μ g/L were associated with severe lung infections [10], indicating that there is a delicate balance between expected therapeutic effect and undesirable side effects, which relate to prednisolone exposure, as measured by cortisol suppression.

Higher prednisolone dose or lower clearance was associated with increased incidence of adverse effects including hyperglycemia, hypertension, and loss of bone density [11]. Side effects of prednisolone at a low dose include fat redistribution, weight gain, suppression of sex hormone secretion, osteoporosis, cushingoid features, skin diseases, aggravation of pre-existing glaucoma, hypertension and difficulty to maintain normoglycemic status in diabetic patients [12],[13]. Apart from generating biochemical imbalance, these side effects often contribute to the lack of adherence to the corticosteroid therapy [14].

Pharmacokinetic characteristics of prednisone or prednisolone are not well studied after transplantation and to date no information is available on the effect of a disease state such as diabetes mellitus on prednisolone pharmacokinetics. Thus we have investigated the pharmacokinetics of total and unbound prednisone and prednisolone in demographically comparable diabetic and nondiabetic stable kidney transplant recipients.

Subjects and Methods

Subjects

Twenty diabetic (7 type 1, 7 type 2, 6 NODAT) and eighteen nondiabetic stable kidney transplant subjects (at least six months post-transplantation with no ongoing episodes of rejection, infection or nephrotoxicity), participating in studies designed to investigate the impact of diabetes on the pharmacokinetics of several immunosuppressant medications, were selected for the present study. Institutional Review Boards of Rhode Island Hospital and of University of Rhode Island approved the studies (IRB # 0159-03, 0054-05 and 2097-08) and the procedure was verbally explained to the participants, who then gave their signed informed consent. The patients were examined by the study physician on the day of the study and found to be eligible for enrolment. Subjects between 18 and 65 years old, of any race and gender were included in the study. Potential subjects were excluded if they were pregnant, nursing, had a pancreas transplant, or if they were taking the oral hypoglycemic agent metformin. All prescribed concomitant medications were continued throughout the study (3 to 21 for diabetic and 3 to 14 for nondiabetic). Apart from insulin and oral hypoglycemic agents that were exclusively taken by diabetics, among the most frequently prescribed for both groups were angiotensin-converting enzyme inhibitors, loop diuretics, proton pump inhibitors, HMG-CoA reductase inhibitors, antifungals, β -blockers, calcium-channel blockers, non-steroidal anti-inflammatory drugs, and antibiotics. In addition to prednisone, the immunosuppressant regimen included mycophenolic acid (either mycophenolate mofetil or enteric-coated mycophenolate sodium) and either tacrolimus, sirolimus, or cyclosporine, at the same dose for 10 days prior to the pharmacokinetics study.

Study Design and Procedures

Following an overnight fast, patients arrived at the hospital at 7:00 AM for a 12-hour pharmacokinetic study and received a physical examination. At approximately 8:00 AM, they were given immunosuppressant medication orally, including prednisone (5 - 10 mg Deltasone[®], Pharmacia and UpJohn Company, Division of Pfizer, New York, NY). As a marker of gastric emptying, 1000 mg acetaminophen (Concentrated Tylenol[®] Infants' Drops; McNeil Consumer and Specialty Pharmaceuticals, Washington, PA) was administered orally with 250 mL of water. Standardized diabetic hospital meals (total intake of 2000 Kcal) were given after approximately 2, 5 and 11 h following dosing. Blood samples were drawn via an intravenous catheter into EDTA Vacutainer[®] tubes (Becton Dickinson, Franklin Lakes, NJ) before and approximately at 0.25, 0.5, 1, 1.5, 2, 3, 5, 7, 9, 10 and 12 h post-dose. Plasma was separated by centrifugation and stored at -80 °C until analysis. Hematology, blood chemistry and urinalysis tests were performed by an accredited national clinical laboratory. Concentration of glycated albumin and corticosteroid binding globulin (CBG) were measured using two commercial Enzyme-Linked ImmunoSorbent

Assay (ELISA) kits: Glycaben® (Exocell Inc., Philadelphia, PA) and Human CBG (BioVendor, Candler, NC), respectively.

Measurement of Total and Free Drugs

Total Glucocorticoids—Total (unbound + bound) concentrations of glucocorticoids were measured in plasma samples using a previously validated LC-MS/MS method over a range of 0.500 – 250 ng/mL for cortisone and prednisone, 1.00 – 500 ng/mL for cortisol, and 2.00 – 1000 ng/mL for prednisolone [15]. In summary, 50 µL of plasma were precipitated with 250 µL of acetonitrile containing deuterated internal standards. The supernatant was evaporated to dryness, reconstituted in 10% acetonitrile in water and 10 µL were injected into LC-MS/MS. Calibration standards and quality control samples were prepared in steroid-free plasma, obtained by repeated clean-up of human plasma with active charcoal.

Free Glucocorticoids—Unbound glucocorticoids were measured using ultrafiltration of samples and online cleanup of the ultrafiltrate followed by LC-MS/MS analysis, as established in a method previously validated in our laboratory over a calibration range of 0.100 to 200 ng/mL for each analyte [16]. The method was slightly modified by performing the equilibration step of 300 µL plasma in open-top ultrafiltration devices for 4 h, at 37 °C, 10% CO₂ and 75% humidity, to insure that differences in pH due to multiple freeze-thaw cycles are corrected.

Acetaminophen—A protein precipitation method was used to extract acetaminophen and the deuterium stable isotope labeled internal standard from 20 µL of plasma. The supernatant diluted with water was injected into LC-MS/MS using a gradient mobile phase containing acetonitrile and water, at a flow of 0.200 mL/min and a HyPURITY™ AquaStar 2x100 mm column (Thermo Scientific, West Palm Beach, FL). Mass spectrometric analysis was performed using electrospray ionization in positive ion mode and multiple reaction monitoring using a triple quadrupole Sciex API 4000 instrument (AB Sciex, Foster, CA). Concentrations of acetaminophen were calculated using a calibration curve with a range of 20.0 – 40,000 ng/mL in human plasma. Accuracy and precision of the method, as measured by quality control samples at three concentrations (60.0, 2,000 and 30,000 ng/mL), were below 9.7% and 5.3%, respectively. No interfering peaks from blank plasma were present in the six blank plasma lots tested prior to sample analysis.

Genotyping for relevant genetic variation—Genomic DNA from patients' peripheral blood sample was extracted using QIAamp DNA Mini Kit (Qiagen, Valencia, CA) according to manufacturer's instructions and was stored at –80°C until analysis. CYP3A4*1B and CYP3A5*3 were genotyped as described previously [17]. Two common polymorphisms in HSD11B1 gene (rs12086634 and rs846910) were determined by TaqMan® allelic discrimination assay (Life Technologies, Foster, CA) using an Applied Biosystems 7500 Real-Time PCR system (Life Technologies) according to manufacturer's instructions.

Pharmacokinetic Analysis—Noncompartmental model 200 (extravascular input) from WinNonlin version 5.2 (Pharsight, Mountain View, CA) was used to determine basic

pharmacokinetic parameters for total and unbound prednisone and prednisolone. This includes area under the concentration-time curve (AUC) from time zero extrapolated to twenty-four hours (AUC_{0-24}), maximum drug concentration in plasma (C_{max}), the corresponding time T_{max} and terminal half-life ($T_{1/2}$). The linear-log trapezoidal method, uniform weighting for λ_z calculations and a log regression with the best fit was used to calculate AUC_{0-24} . Apparent clearance (CL/F) (where F is oral bioavailability), and apparent volume of distribution (V/F) were determined for prednisone. Mean transit time (MTT) was calculated as the ratio between area under the first moment curve (AUMC) and AUC [18]. Prednisone and prednisolone unbound fraction (f_u) were calculated as the ratio between the unbound plasma concentration and the total plasma concentration at each time point.

In plasma, corticosteroids are bound to albumin and CBG. Due to low binding capacity of CBG, cortisol and prednisolone compete for the same binding sites. Therefore, prednisolone plasma protein binding is nonlinear [19] leading to nonlinear pharmacokinetics following higher doses of prednisone [20]. However, dose-normalized exposures of prednisolone are constant, following low oral doses of 10 - 30 mg prednisone [21], therefore, C_{max} and AUC values were normalized by dose.

Statistical Analysis—Statistical analysis of pharmacokinetic parameters was performed with SPSS version 17.0 (SPSS Inc., Chicago, IL). The pharmacokinetic parameters AUC/Dose, C_{max} , $C_{max}/Dose$, MTT, $T_{1/2}$, CL/F and V/F were log-transformed and comparisons between the diabetic and nondiabetic groups were performed using the Student's unpaired *t*-test. Prednisone dose, T_{max} and concentration ratios were compared using Mann-Whitney *U* test. In both cases, a two-sided significance level of 0.05 was used. GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, CA) was used to generate the graphs.

Nonlinear Mixed Effects Modeling of Protein Binding Data—Due to the low binding capacity of CBG, corticosteroids exhibit nonlinear protein binding in plasma. To ascertain the factors contributing to the plasma protein binding of prednisolone, the binding parameters of prednisolone to CBG and albumin were determined using nonlinear mixed effects modeling (NONMEM Version 7.2, ICON Development Group, Hanover, MD) [22] using \$PRED module. First order conditional estimation was used to fit bound and free prednisolone concentrations using the following equation adapted from Rocci et al. [19].

$$PL_B = K_{ALB,PL} * ALB * PL_F + \frac{CBG * K_{CBG,PL} * PL_F}{1 + K_{CBG,PL} * PL_F + K_{CBG,C} * C_F} \quad (\text{Eq. 1})$$

where $K_{ALB,PL}$ is the binding constant for albumin-prednisolone; $K_{CBG,PL}$ and $K_{CBG,C}$ are the affinity constants (assuming one binding site) for CBG-prednisolone and CBG-cortisol, respectively; ALB and CBG are molar concentrations of albumin and CBG, respectively; PL_B , PL_F , and C_F , are molar concentrations of bound prednisolone, free prednisolone and free cortisol, respectively. First-order conditional estimation (FOCE) was used. The model selection was based on goodness-of-fit criteria, including diagnostic plots and minimum objective function value (MOFV). The inter-subject variability in the parameters was

modeled assuming an additive distribution. Residual variability was modeled using an additive model. Covariates from recorded population characteristics such as diabetes, glucose concentration, hemoglobin A1c (HbA_{1c}), creatinine clearance, body mass index (BMI), gender, age and time post transplantation were evaluated by means of stepwise covariate modeling, using the likelihood ratio test with nominal significance of 0.05 for univariate covariate screening and 0.001 for backward elimination (a decrease in minimum objective value of at least 3.84 or 10.83, respectively).

Results

Patient Characteristics

Summary of demographic characteristics and biochemical indices for subjects enrolled in this study are presented in Table 1.

Pharmacokinetic Parameters of Acetaminophen

Acetaminophen is absorbed after reaching the intestine and its concentration-time profile is indicative of the gastric motility [23]. Gastroparesis, a consequence of long-term diabetes, slows the gastric emptying in diabetic patients [24]. The main pharmacokinetic parameters of acetaminophen were calculated using a noncompartmental analysis and compared between the diabetic and nondiabetic groups. Acetaminophen concentrations at 15 and 30 minutes post dose were significantly lower in diabetic subjects ($P < 0.05$). Moreover, the values of AUC from 0 to 1 hour (AUC_{0-1h}) and C_{max} were 25.3% ($P = 0.045$) and 23.2% ($P = 0.015$) lower in diabetic subjects, respectively. Acetaminophen T_{max} expressed as median [25th quartile, 75th quartile] was 0.78 h [0.52 – 1.03] for diabetic and 0.59 h [0.33 – 1.18] for nondiabetic patients ($P = 0.404$). This result indicates a slight delay in the rate of absorption of liquid acetaminophen in patients with diabetes. No subjects received medications that are known to influence gastric emptying, such as erythromycin, metoclopramide or levodopa.

Pharmacokinetic Parameters of Prednisone and Prednisolone

Twelve-hour concentration-time profile of total and free prednisone (PN) and prednisolone (PL) are presented in Figure 1. Pharmacokinetic parameters of total and free prednisone and prednisolone are summarized in Table 2. A delay in T_{max} of approximately 50% was observed for both total and free glucocorticoids in diabetic stable kidney transplant subjects. The delay in the rate of prednisone absorption is concordant with the delay in the rate of acetaminophen absorption. Mean transit time was longer in diabetic patients for total prednisone (14%), total prednisolone (14%), free prednisone (13%) and free prednisolone (15%). Exposure of total and free prednisolone, as measured by dose-normalized AUC, was 27% and 23% higher, respectively, in diabetic subjects than in nondiabetics (Table 2).

Ratio of Prednisolone (PL) Concentration to Prednisone (PN) Concentration

The ratio between total prednisolone to total prednisone concentrations (active/inactive) was 15% higher in diabetic subjects over the 12 -hour post dose ($P < 0.001$); however, the concentration ratios of the corresponding free glucocorticoids were not significantly different between the two groups (Figure 2). Genetic analyses of CYP3A4, CYP3A5 and

HSD11B1 revealed a comparable number of diabetic or nondiabetic patients carrying a polymorphic type of these genes (Table 3) thus excluding the possibility of polymorphism as an underlying factor for higher concentration of prednisolone.

Free Fraction of Prednisone and Prednisolone

Average f_u of prednisone was 31.8% for diabetic subjects and 29.9% for nondiabetic subjects. Overall, prednisone f_u did not differ significantly between diabetic and nondiabetic groups; however, because of non-linear binding to CBG, prednisolone free fraction values increased as the sum of total prednisolone and total cortisol concentrations increased (Figure 3). For total glucocorticoid concentrations below 50 ng/mL, average f_u was 7.1% (range = 4.3 – 10.4%) and it increased to 15.7% (range = 11.0 – 20.4%) for total concentrations above 200 ng/mL.

Factors Affecting Prednisolone Free Fraction

Bound concentration of prednisolone was well described by a nonlinear equation (Eq. 1) as a function of concentrations of free prednisolone, cortisol, CBG and albumin. The mean albumin concentrations were approximately 5% lower ($P < 0.05$) in diabetic patients although within the normal range in both groups. The binding parameters determined using a nonlinear mixed effects model are presented in Table 4. In the covariate screening, diabetes and gender were significant covariates for $K_{CBG,PL}$, while BMI and HbA_{1c} were significant covariates for $K_{CBG,C}$. Moreover, $K_{CBG,PL}$ was 20% higher in diabetic and 22.5% higher in male patients. The effect of BMI and HbA_{1c} on $K_{CBG,C}$ can be described by the following relationship:

$$\text{Typical value of } K_{CBG,C} (\mu M^{-1}) = 66.1 \times (BMI/30)^{2.11} \times (HbA_{1c}/5.8)^{1.96}.$$

Discussion

This is the first study to investigate the impact of diabetes mellitus on the pharmacokinetics of total and unbound prednisone and prednisolone in stable kidney transplant recipients. We have demonstrated that dose-normalized AUC of total and free prednisolone were 27% and 23% higher in the diabetic compared to nondiabetic subjects, respectively. In addition, diabetes influenced the binding of prednisolone to CBG as illustrated by a 20% increase of the affinity constant for CBG-prednisolone ($K_{CBG,PL}$).

In the current study, the time to maximum concentration of prednisone and prednisolone was slightly longer in diabetic patients with no effect on the maximum concentration. Despite this apparent delay, the concentration – time profile of liquid acetaminophen was similar between the two groups of patients.

Higher exposure of prednisolone and a larger prednisolone to prednisone ratio in diabetic patients could be due to (i) increased activity of 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1), which converts prednisone to prednisolone, (ii) reduced inactivation of prednisolone into prednisone by 11 β -hydroxysteroid dehydrogenase type 2, and (iii) reduced

irreversible elimination of prednisolone due to reduced cytochrome P450 (CYP) 3A4 activity [25]. A prospective study in Pima Indians found that higher expression of 11 β -HSD1 mRNA in subcutaneous abdominal adipose tissue at baseline was positively associated with changes in concentration of fasting insulin and homeostatic model assessment (HOMA-IR, a measure of insulin resistance); furthermore, it predicted increases of HOMA-IR after adjustment for covariates [26]. It was found that high glucose concentrations also up-regulate the activity of 11 β -HSD1 [27].

Irreversible elimination of prednisone and prednisolone is partially mediated by CYP 3A enzymes. Diabetes affects the metabolic capacity of the liver, thus possibly resulting in reduced hepatic clearance of prednisolone and higher exposure, as shown by lower fraction of 6- β -hydroxy-prednisolone in urine [28]. *In vitro* studies from our group have also shown a pronounced reduction in CYP 3A4 activity in livers from diabetic subjects [29].

The binding of prednisolone to plasma proteins (CBG and albumin) is nonlinear, and increases with increasing concentrations of corticosteroids [19]. Albumin concentrations were significantly lower in diabetic subjects, possibly leading to higher concentrations of free glucocorticoids; however, in diabetic subjects, exposure of total prednisolone was slightly higher than exposure of free prednisolone (27% vs. 23%). In addition, the average free fractions of prednisone or prednisolone were not different between the two groups, suggesting that this difference in albumin concentrations did not influence the pharmacokinetics of glucocorticoids. Moreover, due to low binding affinity, it was proposed that albumin-bound prednisolone is biologically active [30], hence the lower albumin concentration in diabetic subjects should not influence prednisolone pharmacodynamics.

By the use of nonlinear mixed effect modeling of protein binding parameters, we found that diabetes and gender were significant covariates for the affinity constant of CBG-prednisolone ($K_{CBG,PL}$), diabetic males having the highest affinity constant. However, since only eight females were participating in this study, the influence of gender may not be well characterized by the present analysis. A 20% increase in $K_{CBG,PL}$ may explain the slightly smaller increase in dose-normalized AUC of free prednisolone (23%) than of total prednisolone (27%) in diabetic subjects. Body mass index and HbA_{1c} were significant covariates for the affinity constant of CBG-cortisol ($K_{CBG,C}$), which increased in subjects with higher values of these parameters.

No confounding factors, such as concomitant medications, age, gender, or time post transplantation could account for the higher exposure of prednisolone in diabetic subjects. Transplant recipients participating in this study received numerous concomitant medications, which may contribute to drug-drug interactions. Sulfonylurea, an oral anti-diabetic administered to three diabetic patients, and insulin do not affect prednisolone metabolism. Pioglitazone was taken by one diabetic patients and is listed as an inducer of CYP 3A4. Two diabetic subjects and four nondiabetic subjects were under treatment with the anti-fungal agent, ketoconazole, a known CYP 3A4 inhibitor. Two previous reports show that ketoconazole does not impact the pharmacokinetics of prednisolone [31] [32], while another study suggested interaction resulting in higher exposure of prednisolone [33]. In our study, more nondiabetic subjects were taking ketoconazole, indicating that

ketoconazole did not contribute to our findings of higher prednisolone exposure in diabetic patients. Prednisolone exposure increases slightly with sirolimus dose [34] and in the present study sirolimus dose was not significantly different between the two groups, being marginally higher in nondiabetics. Similarly, the proportion of patients receiving calcineurin inhibitors was slightly higher in diabetics. Diabetes is often accompanied by increased body weight, and glucocorticoids clearance was 10-20% higher in non-obese when related to body weight [35]; however, the mean weight was comparable between diabetic and nondiabetic subjects in this study.

In conclusion, we report for the first time a pharmacokinetics study of prednisone and prednisolone in kidney transplant recipients with diabetes. An increase in prednisolone exposure was observed in stable kidney transplant recipients who have diabetes. Increased exposure to unbound prednisolone in patients with diabetes may result in over-immunosuppression in these patients and thereby may increase the risk of infections. Glucocorticoids are diabetogenic [36], cause peripheral insulin resistance and decreased insulin secretion [37]. The cumulative effect of increase in exposure to unbound prednisolone can potentially worsen glucose control; however, recommendations about a dose adjustment cannot be made in the absence of clinical data correlating plasma concentration of prednisolone with the incidence of side effects. A comprehensive evaluation of the immunosuppressive effect of prednisolone combined with the consideration of undesirable side effects would help to individualize therapy for this population.

Acknowledgments

We acknowledge and thank Dr. Rebecca Boyd who provided feedback and mentorship. Our thanks go to Drs. Joyce Macwan and Shripad Chitnis for their contribution to the clinical study especially with sample collection. We are grateful to Professor Liliana Gonzalez for advice regarding the statistical analysis of data. Partial support of grant # R15GM101599 from National Institutes of Health is gratefully acknowledged.

References

1. Venkataramanan R, Habucky K, Burckart GJ, et al. Clinical pharmacokinetics in organ transplant patients. *Clin Pharmacokinet*. 1989; 16:134–61. [PubMed: 2656047]
2. Pascual J, Quereda C, Zamora J, et al. Steroid withdrawal in renal transplant patients on triple therapy with a calcineurin inhibitor and mycophenolate mofetil: a meta-analysis of randomized, controlled trials. *Transplantation*. 2004; 78:1548–56. [PubMed: 15599321]
3. Akhlaghi F, Dostalek M, Falck P, et al. The concentration of cyclosporine metabolites is significantly lower in kidney transplant recipients with diabetes mellitus. *Ther Drug Monit*. 2012; 34:38–45. [PubMed: 22210099]
4. Patel CG, Richman K, Yang D, et al. Effect of diabetes mellitus on mycophenolate sodium pharmacokinetics and inosine monophosphate dehydrogenase activity in stable kidney transplant recipients. *Ther Drug Monit*. 2007; 29:735–42. [PubMed: 18043470]
5. Akhlaghi F, Patel CG, Zuniga XP, et al. Pharmacokinetics of mycophenolic acid and metabolites in diabetic kidney transplant recipients. *Ther Drug Monit*. 2006; 28:95–101. [PubMed: 16418701]
6. Kuo HT, Sampaio MS, Vincenti F, et al. Associations of pretransplant diabetes mellitus, new-onset diabetes after transplant, and acute rejection with transplant outcomes: an analysis of the Organ Procurement and Transplant Network/United Network for Organ Sharing (OPTN/UNOS) database. *Am J Kidney Dis*. 2010; 56:1127–39. [PubMed: 20934793]

7. Ramezani M, Ghoddousi K, Hashemi M, et al. Diabetes as the cause of end-stage renal disease affects the pattern of post kidney transplant rehospitalizations. *Transplant Proc.* 2007; 39:966–9. [PubMed: 17524864]
8. Frey BM, Walker C, Frey FJ, et al. Pharmacokinetics and pharmacodynamics of three different prednisolone prodrugs: effect on circulating lymphocyte subsets and function. *J Immunol.* 1984; 133:2479–87. [PubMed: 6237151]
9. Russell GM, Henley DE, Leendertz J, et al. Rapid glucocorticoid receptor-mediated inhibition of hypothalamic-pituitary-adrenal ultradian activity in healthy males. *J Neurosci.* 2010; 30:6106–15. [PubMed: 20427668]
10. Oka K, Hirano T, Shimodaira H, et al. Suppression of endogenous cortisol for evaluating pharmacodynamics of prednisolone in early allograft rejection in renal transplantation. *Clin Chem.* 1990; 36:481–6. [PubMed: 2311218]
11. Kozower M, Veatch L, Kaplan MM. Decreased clearance of prednisolone, a factor in the development of corticosteroid side effects. *J Clin Endocrinol Metab.* 1974; 38:407–12. [PubMed: 4815171]
12. Citterio F. Steroid side effects and their impact on transplantation outcome. *Transplantation.* 2001; 72:S75–80. [PubMed: 11833145]
13. Da Silva JA, Jacobs JW, Kirwan JR, et al. Safety of low dose glucocorticoid treatment in rheumatoid arthritis: published evidence and prospective trial data. *Ann Rheum Dis.* 2006; 65:285–93. [PubMed: 16107513]
14. Schweizer RT, Rovelli M, Palmeri D, et al. Noncompliance in organ transplant recipients. *Transplantation.* 1990; 49:374–7. [PubMed: 2305467]
15. Ionita IA, Fast DM, Akhlaghi F. Development of a sensitive and selective method for the quantitative analysis of cortisol, cortisone, prednisolone and prednisone in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2009; 877:765–72.
16. Ionita IA, Akhlaghi F. Quantification of unbound prednisolone, prednisone, cortisol and cortisone in human plasma by ultrafiltration and direct injection into liquid chromatography tandem mass spectrometry. *Ann Clin Biochem.* 2010; 47:350–7. [PubMed: 20592334]
17. Chitnis SD, Ogasawara K, Schniedewind B, et al. Concentration of tacrolimus and major metabolites in kidney transplant recipients as a function of diabetes mellitus and cytochrome P450 3A gene polymorphism. *Xenobiotica.* 2013; 43:641–9. [PubMed: 23278282]
18. Rosenbaum, S. Basic pharmacokinetics and pharmacodynamics: an integrated textbook and computer simulations. Hoboken, N.J.: John Wiley & Sons; 2011.
19. Rocci ML Jr, D'Ambrosio R, Johnson NF, et al. Prednisolone binding to albumin and transcortin in the presence of cortisol. *Biochem Pharmacol.* 1982; 31:289–92. [PubMed: 7073761]
20. Wald JA, Law RM, Ludwig EA, et al. Evaluation of dose-related pharmacokinetics and pharmacodynamics of prednisolone in man. *J Pharmacokinet Biopharm.* 1992; 20:567–89. [PubMed: 1302763]
21. Uribe M, Schalm SW, Summerskill WH, et al. Oral prednisone for chronic active liver disease: dose responses and bioavailability studies. *Gut.* 1978; 19:1131–5. [PubMed: 744499]
22. Boekmann, A. NONMEM User's Guide, Part V: Introductory Guide. In: Sheiner, L., editor. Technical Report of the Division of Clinical Pharmacology. University of California; San Francisco: 1992.
23. Maddern G, Miners J, Collins PJ, et al. Liquid gastric emptying assessed by direct and indirect techniques: radionuclide labelled liquid emptying compared with a simple paracetamol marker method. *Aust N Z J Surg.* 1985; 55:203–6. [PubMed: 3862394]
24. Soykan I, Sivri B, Sarosiek I, et al. Demography, clinical characteristics, psychological and abuse profiles, treatment, and long-term follow-up of patients with gastroparesis. *Dig Dis Sci.* 1998; 43:2398–404. [PubMed: 9824125]
25. Garg V, Jusko WJ. Bioavailability and reversible metabolism of prednisone and prednisolone in man. *Biopharm Drug Dispos.* 1994; 15:163–72. [PubMed: 8011967]
26. Koska J, de Courten B, Wake DJ, et al. 11 β -hydroxysteroid dehydrogenase type 1 in adipose tissue and prospective changes in body weight and insulin resistance. *Obesity (Silver Spring).* 2006; 14:1515–22. [PubMed: 17030962]

27. Fan Z, Du H, Zhang M, et al. Direct regulation of glucose and not insulin on hepatic hexose-6-phosphate dehydrogenase and 11 β -hydroxysteroid dehydrogenase type 1. *Mol Cell Endocrinol*. 2011; 333:62–9. [PubMed: 21163329]
28. Renner E, Horber FF, Jost G, et al. Effect of liver function on the metabolism of prednisone and prednisolone in humans. *Gastroenterology*. 1986; 90:819–28. [PubMed: 3512355]
29. Dostalek M, Court MH, Yan B, et al. Significantly reduced cytochrome P450 3A4 expression and activity in liver from humans with diabetes mellitus. *Br J Pharmacol*. 2011; 163:937–47. [PubMed: 21323901]
30. Lentjes EG, Romijn FH. Temperature-dependent cortisol distribution among the blood compartments in man. *J Clin Endocrinol Metab*. 1999; 84:682–7. [PubMed: 10022438]
31. Yamashita SK, Ludwig EA, Middleton E Jr, et al. Lack of pharmacokinetic and pharmacodynamic interactions between ketoconazole and prednisolone. *Clin Pharmacol Ther*. 1991; 49:558–70. [PubMed: 1827622]
32. Jeng S, Chanchairujira T, Jusko W, et al. Prednisone metabolism in recipients of kidney or liver transplants and in lung recipients receiving ketoconazole. *Transplantation*. 2003; 75:792–5. [PubMed: 12660503]
33. Zurcher RM, Frey BM, Frey FJ. Impact of ketoconazole on the metabolism of prednisolone. *Clin Pharmacol Ther*. 1989; 45:366–72. [PubMed: 2639662]
34. Jusko WJ, Ferron GM, Mis SM, et al. Pharmacokinetics of prednisolone during administration of sirolimus in patients with renal transplants. *J Clin Pharmacol*. 1996; 36:1100–6. [PubMed: 9013365]
35. Milsap RL, Plaisance KI, Jusko WJ. Prednisolone disposition in obese men. *Clin Pharmacol Ther*. 1984; 36:824–31. [PubMed: 6499362]
36. Delaunay F, Khan A, Cintra A, et al. Pancreatic beta cells are important targets for the diabetogenic effects of glucocorticoids. *J Clin Invest*. 1997; 100:2094–8. [PubMed: 9329975]
37. Lambillotte C, Gilon P, Henquin JC. Direct glucocorticoid inhibition of insulin secretion. An in vitro study of dexamethasone effects in mouse islets. *J Clin Invest*. 1997; 99:414–23. [PubMed: 9022074]

Abbreviations

11β-HSD1	11 β -hydroxysteroid dehydrogenase type 1
11β-HSD2	11 β -hydroxysteroid dehydrogenase type 2
AUC	area under the concentration-time curve
AUC₀₋₂₄	AUC from time zero extrapolated to twenty-four hours
BMI	body mass index
CBG	corticosteroid binding globulin
CL/F	apparent clearance
C_{max}	maximum drug concentration in plasma
ELISA	Enzyme-Linked Immunosorbent Assay
F	oral bioavailability
f_u	unbound fraction
HbA_{1c}	hemoglobin A _{1c}
MTT	mean transit time

NODAT	new-onset diabetes after transplantation
NONMEM	nonlinear mixed effects modeling
T_{1/2}	elimination half-life
T_{max}	the time corresponding to C _{max}
V/F	apparent volume of distribution

Figure 1.

Geometric mean of dose-normalized plasma concentrations of (A) total prednisone, (B) total prednisolone, (C) free prednisone and (D) free prednisolone versus time following oral administration of prednisone (dose ranging from 5 to 10 mg administered in the morning). Error bars represent the upper or the lower 95% confidence interval of the geometric mean. Closed circles – diabetic subjects. Open circles – nondiabetic subjects.

Figure 2. Prednisolone to prednisone ratio for total (A) and free (B) concentrations in diabetic and nondiabetic subjects. Results expressed as median and interquartile range and outliers. P values were calculated using Mann-Whitney U test.

Figure 3. Median values of total cortisol (grey bars), total prednisolone (white bars) and unbound fraction (f_u) of prednisolone (line) versus time post dose in diabetic (A) and nondiabetic (B) subjects.

Table 1
Summary of demographic characteristics and biochemical indices

Characteristic	Diabetic (N=20)	Nondiabetic (N=18)
Gender, Male	80.0%	77.8%
Race, Caucasian	90% (1 African American, 1 Hispanic)	67% (3 African American, 2 Hispanic, 1 Asian)
Geometric mean [95% CI]		
Age (years)	51.3 [46.1, 57.2]	50.2 [43.9, 57.5]
Weight (kg)	87.0 [78.2, 96.8]	86.2 [78.0, 95.1]
Body mass index	29.2 [27.1, 31.6]	30.1 [27.2, 33.3]
Post transplantation duration (months)	34.3 [22.6, 51.9]	30.8 [20.5, 46.3]
Prednisone dose (mg/day)	5.41 [4.89, 5.98]	5.88 [5.14, 6.73]
Weight adjusted prednisone dose (mg/kg/day)	0.0622 [0.0530, 0.0729]	0.0683 [0.0566, 0.0823]
Cyclosporine dose (mg/day); N	55.9; N=2	55.3; N=4
Tacrolimus dose (mg/day); N	2.91 [1.83, 4.61] N=10	4.42 [2.99, 6.53] N=5
MPA dose (mg/day)	1170 [1004, 1363]	1197 [865, 1658]
Sirolimus dose (mg/day); N	1.77 [1.29, 2.43] N=8	2.29 [1.68, 3.12] N=9
Serum creatinine (mg/dL)	1.47 [1.31, 1.66]	1.40 [1.12, 1.75]
Creatinine clearance (mL/min) ^a	52.9 [50.0, 60.9]	53.0 [41.9, 66.9]
BUN (mg/dL)	27.6 [22.5, 33.9]	22.5 [19.0, 26.6]
HbA _{1c} (%)	7.7 [6.8, 8.6] ^b	5.4 [5.2, 5.7]
Fasting glucose (mg/dL)	135 [106, 170] ^b	86.8 [79.1, 95.3]

^aEstimated using Cockcroft-Gault equation.

^bP < 0.01

N = Number of patients

Table 2
Pharmacokinetic parameters of total and free prednisone and prednisolone

Parameter	Diabetic Subjects (n=20)	Nondiabetic Subjects (n=18)	Ratio	P
Total Prednisone				
T _{max} (h)	3.08 [2.36, 4.80]	2.13 [1.5, 4.13]	1.45	0.04
C _{max} (µg/L)	12.4 [10.6, 14.5]	13.6 [11.4, 16.2]	0.91	0.43
C _{max} /Dose (µg/L/mg)	2.29 [1.97, 2.66]	2.31 [1.92, 2.78]	0.99	0.95
AUC ₀₋₄ /Dose(µg*h/L/mg)	20.3 [17.9, 23.1]	17.9 [15.0, 21.2]	1.14	0.21
MTT ₀₋₂₄ (h)	7.10 [6.59, 7.65]	6.21 [5.74, 6.73]	1.14	0.02
T _{1/2} (h)	3.62 [3.11, 4.20]	3.25 [2.84, 3.72]	1.11	0.28
CL/F (L/h/Kg)	0.551 [0.483, 0.629]	0.641 [0.544, 0.754]	0.86	0.14
V/F (L/Kg)	2.88 [2.40, 3.45]	3.01 [2.44, 3.70]	0.96	0.74
Total Prednisolone				
T _{max} (h)	1.85 [1.18, 2.88]	1.26 [0.560, 2.20]	1.47	0.09
C _{max} (µg/L)	149 [126, 175]	150 [133, 170]	0.99	0.93
C _{max} /Dose (µg/L/mg)	27.6 [23.9, 31.7]	25.6 [22.3, 29.3]	1.08	0.43
AUC ₀₋₄ /Dose(µg*h/L/mg)	208 [186, 233]	164 [141, 192]	1.27	0.01
MTT ₀₋₂₄ (h)	6.42 [5.98, 6.88]	5.65 [5.20, 6.14]	1.14	0.02
T _{1/2} (h)	3.87 [3.40, 4.21]	3.72 [3.41, 4.05]	1.04	0.80
Free Prednisone				
T _{max} (h)	3.00 [2.05, 4.08]	2.04 [1.30, 2.20]	1.47	0.02
C _{max} (µg/L)	4.09 [3.53, 4.75]	4.34 [3.76, 5.01]	0.94	0.55
C _{max} /Dose (µg/L/mg)	0.757 [0.655, 0.874]	0.738 [0.621, 0.877]	1.03	0.82
AUC ₀₋₄ /Dose(µg*h/L/mg)	6.31 [5.64, 7.06]	5.33 [4.53, 6.27]	1.18	0.08
MTT ₀₋₂₄ (h)	6.73 [6.22, 7.29]	5.97 [5.49, 6.49]	1.13	0.04
T _{1/2} (h)	3.49 [3.14, 3.88]	3.29 [2.86, 3.77]	1.06	0.48
CL/F (L/hr/Kg)	1.78 [1.57, 2.03]	2.15 [1.80, 2.57]	0.83	0.20
V/F (L/Kg)	8.97 [7.91, 10.2]	10.2 [8.66, 12.0]	0.88	0.08
Free Prednisolone				
T _{max} (h)	1.80 [1.04, 2.44]	1.01 [0.558, 1.65]	1.78	0.01
C _{max} (µg/L)	22.3 [18.0, 27.4]	22.9 [19.6, 26.9]	0.97	0.81
C _{max} /Dose (µg/L/mg)	4.11 [3.46, 4.90]	3.90[3.25, 4.68]	1.37	0.66
AUC ₀₋₄ /Dose(µg*h/L/mg)	21.7 [19.5, 24.1]	17.7 [15.2, 20.4]	1.23	0.02
MTT ₀₋₂₄ (h)	5.16 [4.72, 5.65]	4.47 [4.09, 4.89]	1.15	0.02
T _{1/2} (h)	3.12 [2.90, 3.36]	2.83 [2.63, 3.04]	1.10	0.05

Results expressed as geometric mean [95% confidence interval] except for $T_{\max}$ values that are expressed as median [25th quartile, 75th quartile].
Dose of prednisone was used for dose normalization.

Table 3
Comparison of genetic polymorphism of *CYP3A4*, *CYP3A5* or 11 β -hydroxysteroid dehydrogenase type 1 (*HSD11B1*) between diabetic and nondiabetic patients

		Diabetic	Nondiabetic
<i>CYP3A4</i> *1/*1) / (*1/*1B + *1B/*1B)	rs2740574	17/3	15/3
<i>CYP3A5</i> expresser/non-expresser*	rs776746	3/17	4/14
<i>HSD11B1</i> TT / TG+GG	rs12086634	16/4	11/7
<i>HSD11B1</i> GG / GA+AA	rs846910	17/3	16/2

* *CYP3A5* expresser: *CYP3A5**1/*1 + *1/*3, *CYP3A5* non-expresser: *CYP3A5**3/*3

Table 4
Concentration of binding proteins, corticosteroid binding globulin (CBG), albumin and glycated albumin in diabetic and nondiabetic kidney transplant recipients (top part); binding parameters of prednisolone and cortisol to CBG and albumin obtained by fitting prednisolone and cortisol concentrations into the equation proposed by Rocci et al.[19] using NonLinear Mixed Effects Modeling

Parameter	Diabetic (N=20)	Nondiabetic (N=18)
Geometric mean [95% CI]		
CBG concentration (μM)	0.490 [0.454, 0.531]	0.467 [0.433, 0.506]
Albumin concentration (μM)	613 [591, 637] ^a	645 [640, 663]
Glycated albumin (%)	1.44 [1.12, 1.86]	1.37 [1.05, 1.79]
Estimate (%RSE)		
$K_{\text{CBG,PL}}$ (μM^{-1})	Male	33.1 (4.0) ^{a, b}
	Female	25.7 (6.4) ^a
$K_{\text{CBG,C}}$ (μM^{-1})	66.1 (15.3)	
$K_{\text{ALB,PL}}$ (μM^{-1})	0.00301 (9.5)	

$K_{\text{CBG,PL}}$, affinity constant for CBG-prednisolone

$K_{\text{CBG,C}}$, affinity constant for CBG-cortisol

$K_{\text{ALB,PL}}$, binding constant for albumin-prednisolone

^aSignificantly different from nondiabetic ($P < 0.05$)

^bSignificantly different from female ($P < 0.05$)