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Supplementary Materials

Clinical Study Designs
Both clinical studies were conducted in healthy subjects and at a single clinical research unit. ASEA used a randomized, double-blind, placebo-controlled, dose-escalating, incomplete crossover design in 3 groups of 9 subjects each, for a total of 27 subjects enrolled (Supplementary Table S1a). The first 2 groups were dosed in an alternating fashion during 3 separate admission periods. The third group was dosed during 2 separate admission periods. Study drug was administered as an oral solution in degassed Sprite on an in-clinic basis in the fed state at half-log dose increments across the dose range of 0.1 mg to 300 mg. Subjects were randomized 2:1 for LY3045697 to placebo in each dosing group with each subject receiving placebo in a maximum of 1 period. There was a planned washout period of at least 5 days between periods for an individual subject. The actual washout period ranged from 7-14 days for individual subjects.

ASEB used a randomized, double-blind, placebo and positive comparator (spironolactone) controlled, multiple dose escalating, incomplete cross-over design in 24 subjects, divided into 2 groups of 12 subjects each, which were dosed in an alternating fashion during 3 separate admission periods per group (Supplemental Table S1b). Subjects were housed at the clinical research facility for the duration of dosing in each study period. Study drug was administered once daily in the fed state for 8 consecutive days. Within each period, subjects were randomized 4:1:1 to LY3045697:placebo:spironolactone, and by the end of the study each subject had received LY3045697 in two dosing periods and either placebo or spironolactone in one dosing period.

Table S1a. ASEA Study

Study Diagram									
		Period 1		Period 2		Period 3		Follow-Up	
Group 1 (N=9)	Screening	Dose 1	≥ 5 days	Dose 3	≥ 7 days	Dose 5	~7- 10 days	FU visit	
Group 2 (N=9)	Screening		Dose 2	≥ 5 days	Dose 4	≥ 5 days	Dose 6	~7- 10 days	FU Visit
Group 3 (N=9)	Screening	Dose 7	≥ 5 days	Dose 8	~7- 10 days			Follow Up Visit	

 *Within each treatment period, subjects will receive either LY3045697 or placebo at a ratio of 2:1

Table S1b. ASEB Study

Study Diagram									
		Period 1		Period 2		Period 3		Follow-Up	
Group 1 (N=12)	Screening	Dose 1*	≥ 7 days	Dose 3*	≥ 7 days	Dose 1 or 3*	~7- 10 days	FU visit	
Group 2 (N=12)	Screening		Dose 2*	≥ 7 days	Dose 4*	≥ 7 days	Dose 2 or 4*	~7- 10 days	FU Visit

 *Within each treatment period, subjects will receive either LY3045697 or placebo or spironolactone at a ratio of 4:1:1

Subjects

Healthy male or female subjects were eligible and had to provide written, informed consent prior to starting any study procedures. Females had to be postmenopausal or surgically sterile to participate. Subjects had to have venous access sufficient to allow blood sampling as per the protocol, be a non-smoker, and agree to refrain from certain foods or beverages.

Subjects were excluded from the study if they had a history or presence of medical illness that in the judgment of the PI, indicates a medical problem that would preclude study participation.

Pharmacokinetic Sampling

ASEA

In study ASEA, PK blood sampling for plasma LY3045697 occurred pre-dose and at the following time points post-dose: 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 hours. In addition, a PK blood sample was collected at the follow-up visit which occurred 7-10 days after the last dose (last study period) per subject.

ASEB

In study ASEB, PK blood sampling for plasma LY3045697 occurred pre-dose on Days 1, 3, 8; and on Day 8 at 0.5, 1, 2, 3, 4, 6, 8, 12, 24 h and 48 h post-dose. In addition, a PK blood sample was collected at the follow-up visit which occurred 7-10 days after the last dose (last study period) per subject.

Safety Analyses

In study ASEA, subjects were confined to the CRU from the evening prior to dosing until 72 hours after dosing in each dosing period. In study ASEB, subjects were confined to the CRU from the evening prior to dosing until 48 hours after completion of dosing in each dosing period. Medical supervision and oversight was conducted at all times. Safety evaluations and analyses included physical examinations, scheduled vital signs, 12-lead electrocardiograms (ECGs), clinical chemistry, hematology and urinalysis collections and analyses, as well as recording and follow up of adverse events.

Mean Arterial Blood Pressure

Mean arterial pressure diurnal profiles were generated based on duplicate blood pressure measured on Day 3 and 7 at pre-dose and at 4, 8, 12, and 24 hours after dosing (24-hour measures were prior to next daily dose). Blood pressure was measured with the forearm passively supported at the level of the heart with an appropriate sized sphygmomanometer cuff. The study environment was non-stressful and phlebotomy was not performed within 10 minutes prior to blood pressure measurement.

Mean arterial pressure (MAP) was calculated from diastolic and systolic blood pressure according to the following formula:

- MAP = diastolic blood pressure (DBP) + ⅓ (systolic blood pressure [SBP]-DBP).

Duplicate measurements of DBP and SBP were averaged at each time point before any calculation.

Oral potassium challenge
The oral K^+ challenge was administered to subjects on Day 7 in each dosing period in study ASEB. Study drug was administered after breakfast on Day 7, 3 hours prior to administration of the oral K^+ bolus which consisted of 40 mEq potassium (10% potassium chloride in 30 mL volume). The potassium content of food on Day 7 was reduced by 40 mEq, to maintain total daily K^+ intake of 125 mEq/day.

Serum for potassium measures was collected during each period at -1.5 h (before K^+ administration) and at 0, 1, 2, 3, 4 and 5 h relative to the oral K^+ administration on Day 7. Urine fractions were collected for K^+ and Na^+ measurements on Day 7 at the following time intervals relative to the oral K^+ administration: -3 to 0 h; 0 h to 5 h. Potassium chloride solution used for the oral challenge was manufactured by PRA pharmacy.

IV ACTH challenge on Day 8
During each period, an IV ACTH challenge was conducted on Day 8. ACTH (250 µg) was administered IV as a bolus 1 hour post-dose. Plasma samples for the analysis of aldosterone, corticosterone, 11-deoxycorticosterone, cortisol and 11-deoxycortisol (5-steroid panel) relative to the ACTH challenge were collected 15 minutes before and 30 and 60 minutes after administration of IV ACTH. ACTH (Synacthen®) was manufactured by Defiante Farmaceutica, S.A. A 1 mL injection volume at 0.25 mg/mL tetracosactide hexa acetate was administered per challenge.

The studies were conducted by PRA Heath Sciences, in accordance with the current version of the Declaration of Helsinki and in compliance with current regulations and standards of Good Clinical Practice (GCP). All volunteers signed a written Informed Consent Form.

Bioanalytical Methods

A bioanalytical method was validated for the determination of aldosterone in human urine by liquid chromatography atmospheric pressure ionization tandem mass spectrometry (LC-MS/MS). Synthetic urine was used as a surrogate matrix. Aldosterone-d_7 was used as the internal standard.

A bioanalytical method was validated for the determination of cortisol and corticosterone in human plasma (K_2EDTA) by LC-MS/MS. Dialyzed 3% BSA was used as a surrogate matrix, due to the presence of endogenous analyte levels in human plasma. Hydrocortisone-d_4 and corticosterone-d_8 were used as the internal standards.

A bioanalytical method was validated for the determination of aldosterone and 11-deoxycortisol and qualified for the determination of 11-deoxycorticosterone in human plasma (K_2EDTA) by LC-MS/MS. Precision in the endogenous QC samples for 11-deoxycorticosterone did not meet validation specifications; hence the method is considered qualified. Dialyzed 2% bovine serum albumin (BSA) was used as a surrogate matrix, due to the presence of endogenous analyte in human plasma. Aldosterone

d$_7$, 11-deoxycortisol-d$_5$, and cortexone-d$_8$ were used as the internal standards for aldosterone, 11-deoxycortisol, and 11-deoxycorticosterone, respectively.

Plasma renin activity is a measure of the activity of the plasma enzyme renin, which plays a major role in the regulation of blood pressure. The enzyme is the first step in the renin-angiotensin-aldosterone cycle. This cycle includes alpha-2 globulin cleaved by plasma renin resulting in angiotensin I, which in turn is converted to angiotensin II. Angiotensin II is a powerful blood vessel constrictor, and causes contraction of the arteriolar and renal vascular smooth muscle, leading to retention of water and salt in the kidney and increased arterial blood pressure. In addition, angiotensin II stimulates the release of aldosterone from the adrenal cortex, which in turn also increases water and salt retention in the kidney. Angiotensin II has an extremely short half-life in vivo, resulting in challenges with its analytical measurement. Since angiotensin I levels are a direct representation of plasma renin activity, the determination of plasma renin activity has been adopted to evaluate renin-angiotensin system in diseased states. A Radioimmunoassay Kit (GammaCoat [^{125}I] Plasma Renin Activity Radioimmunoassay Kit, DiaSorin, Cat. # 2733) was used to quantify generated angiotensin I after an incubation of the plasma to generate angiotensin I.

The above analytical methods and sample analyses were conducted by Intertek Pharmaceutical Services in CA.

LY3045697 was quantified in human plasma using a High Performance Liquid Chromatograph with Tandem Mass Spectrometric Detection with a calibration range of 0.05 − 100 ng/mL. This method was developed and validated, and samples analyzed at PRA Netherlands Bioanalytical Laboratory.

PK/PD and Modeling Methods

The relationship between drug exposure and effects on plasma aldosterone concentration (PAC), urine aldosterone excretion and K$^+$ responses in the different experimental contexts was characterized quantitatively through dose and concentration-response models. All such models used simple hyperbolic functions (E$_{max}$, I$_{max}$) with dose and plasma LY3045697 concentration as the predictor. Such models assume a direct relationship between plasma drug concentration and PD response. Assessment of hysteresis and more indirect PK/PD models were attempted where appropriate.

Concentration-response models used plasma LY3045697 concentration that associated temporally with the PD observation. When a LY3045697 concentration was not measured at the time of the PD observation, an imputation was conducted. For example, plasma drug concentration was not measured on Day 7 of study ASEB, when aldosterone was measured. Observed plasma LY3045697 concentration on Day 7 was used to impute the concentrations on Day 8, which assumes steady state was achieved, an assumption that was consistent with the short half-life (mean terminal half-life ranged between 9.8-10.8 h on Day 8 of Study ASEB) and observed trough levels over time. Where an imputation from an alternate observed drug plasma concentration was not possible, values were imputed as the prediction from a population PK model that was developed to describe the pharmacokinetics of LY3045697 upon multiple dose administration. The model was a typical two-compartment model with first order absorption, parameterized using macro constants. Predicted concentration values for each individual at the corresponding times of PD observations were then generated using Bayesian post hoc procedures, which utilizes all observations from the individual and the population to predict the individual PK parameters and the concentration-time curve. The lower limit of quantitation (LLOQ) for LY3045697 plasma concentrations was 0.05 ng/mL. Samples (11% of total) with concentrations below quantitation limit (BQL; 0.05 ng/mL) were imputed at 0.01 ng/mL, which resulted in the minimum objective function when different imputations were explored.

To facilitate joint estimates and predictions of K^+ and aldosterone responses, model fitting was conducted in pairs (one aldosterone and one K^+ endpoint) simultaneously fitted at a time. Visualizations of responses at different LY3045697 dose or plasma concentrations were generated through simulations from the model fits, utilizing the variance-covariance matrix of parameter estimates as a multivariate normal distribution. Random effects (inter-individual and residual variability) were ignored in these simulations, representing the response in the typical individual.

Concentration Response Modeling Methods

The concentration response models took one of the following general forms:

$$A_{ij} = (\text{placebo} + \Delta SPN)\, e^{\eta_{Ai}}\ [\ 1 - I_{max}\{\ C_{ij}/(C_{ij} + IC_{50})\ \}\ ],\ \text{or} \tag{1}$$

$$P_{ij} = (\text{placebo} + \Delta SPN)\, e^{\eta_{Pi}}\ [\ E_{max}\{\ C_{ij}/(C_{ij} + EC_{50})\ \}\ ], \tag{2}$$

$$i=1, \ldots, 24 \text{ and } j=1, \ldots, 4 \tag{3}$$

where A_{ij} and P_{ij} are the ith aldosterone and potassium observations in the jth subject, respectively, placebo *is the estimated aldosterone or potassium under placebo treatment, and ΔSPN is the estimated* difference due to 25 mg spiranolactone. ΔSPN is set to zero when treatment is placebo or LY3045697. I_{max} and E_{max} are the maximum LY3045697-associated reduction in aldosterone and increase in potassium (at a sufficiently high dose), respectively, while IC_{50} and EC_{50} are the time-matched LY3045697 plasma concentration associated with 50% of *Imax or Emax, respectively*. The η_{Ai} and η_{Pi} is subject *i* random effect, assumed normally distributed with a mean of 0.

Where aldosterone or potassium was measured over time, time dependencies were observed in aldosterone levels during the day, unperturbed by LY3045697, as well as fasting potassium levels across days of continuous dosing. For these, the following variation of the model was adopted:

$$A_{ij}(t)= (\text{placebo} + \beta_t + \Delta SPN)\, e^{\eta_{Ai}}\ [\ 1 - \text{Imax}\{\ C_{ij}/(C_{ij} + IC_{50}(t)\ )\ \}\ ],\ t=0, 4, 12 \text{ or } 24\ hr \tag{1}$$

$$P_{ij}(\text{day})= (\text{placebo} + \tau_1\, \text{day} + \tau_2\, \text{day}^2 + \Delta SPN)\, e^{\eta_{Pi}}\ [\ e_{max}\, \text{day}\ \{\ C_{ij}/(C_{ij} + EC_{50})\ \}\ ],\ \text{day}= 1, 3, 7 \text{ or } 8 \tag{2}$$

For aldosterone, β_t represents the estimated difference in aldosterone at t= 4, 12 or 24 hours, compared to the zero hour, and τ_1 and τ_2 are the second-order polynomial function parameters relating placebo response to time. In this model, the E_{max} of K^+ is assumed to increase linearly with time. Although this is unlikely if dosing was continued beyond 14 days, in our study no plateauing was observed in maximum stimulation of serum potassium.

Linear and proportional residual error models were attempted.

Direct vs. Indirect PK/PD relationship

Basal plasma aldosterone was collected a few times within the day in ASEA and ASEB. The hyperbolic functions mentioned above imply a direct relationship between drug exposure and the PD endpoints. To test this assumption, a couple of procedures were implemented. First, the temporal relationship between drug plasma concentration and basal plasma aldosterone was explored through hysteresis plots (ref), where the time-matched LY3045697 plasma concentration and aldosterone points were joined temporally. Second, a delay in the PK/PD relationship was also tested through a PK/PD model, where a delay between the central compartment (plasma) and an effect compartment was introduced, and the delay time constant was estimated to quantify the magnitude of deviation from the direct PK/PD relationship.

Imputations

Concentration-response models used plasma LY3045697 concentration that associated temporally with the PD observation. When a LY3045697 concentration was not measured at the time of the PD observation, an imputation was conducted. For example, plasma drug concentration was not measured on Day 7 of study ASEB, when aldosterone was measured, and were imputed as observed drug plasma concentrations on Day 8, assuming steady state was achieved, which is supported by the data and the mean terminal half-life which ranged between 9.8-10.8 hours on Day 8 of Study ASEB. Where an imputation from an alternate observed drug plasma concentration was not possible, values were imputed as the prediction from a population PK model that was developed to describe the pharmacokinetics of LY3045697 upon multiple dose administration. The model is described below.

A significant portion of PAC data especially at high LY3045697 dose or concentration were below the limit of quantitation (10 pg/mL) due to a high degree of inhibition These represent 25% of the basal PAC data after single dose administration and 5% after multiple dose administration, and 11% of post-ACTH challenge PAC. These values were imputed at half the quantitation limit. A sensitivity analysis showed a marginal effect of this imputation on parameter estimates.

Population PK Modeling Results
A two-compartment model was found appropriate, and was parameterized using macro constants, alpha, beta (the bi-exponential decay parameters) and K21, the transfer rate constant between the second (peripheral) compartment and the first (central) compartment. The model incorporated subject random in absorption rate Ka, alpha and in apparent volume of distribution in the central compartment (V/F). To avoid over-parameterization no random effects were incorporated in Beta and K21. The standard deviations of the random effects (SD eta), which are approximately equal to coefficient of variation is small for V/F, suggesting small overall inter-subject variability in exposure when receiving the same dose. The residual error model is proportional with SD=$C_{pred}{}^{\delta}.\sigma$. For example a LY3045697 0.3 mg dose is estimated to give peak concentration of 1 ng/mL. At this concentration, the residual SD is 0.285. For LY3045697 3 mg peak concentration is 10 ng/mL, giving a SD of $10^{0.824}$ x 0.285=1.9 ng/mL. At 100 ng/mL SD=12.7 ng/mL. Model parameter estimates as shown in Supplementary Materials Table S3. The model showed very good congruence with observed concentrations (See Figure S1 below and Figure 2).

Table S3. Two-compartment Population Pharmacokinetic Model Parameter Estimate

Parameter	Estimate	%CV
Ka (1/H)	1.82	14.1
Alpha (1/H)	0.47	5.9
Beta (1/H)	0.06	3.1
K21 (1/H)	0.09	4.9
V/F (L)	181	5.5
IIV Ka	0.58	
IIV alpha	0.17	
IIV V/F	0.2	
Delta	0.82	
Error	0.28	

Subjects = 24; # Observations = 528; Log Lik. = -508.43

beta = the bi-exponential decay parameters; K21 = the transfer rate constant between the second (peripheral) compartment and the first (central) compartment; Ka = absorption rate; V/F = apparent volume of distribution
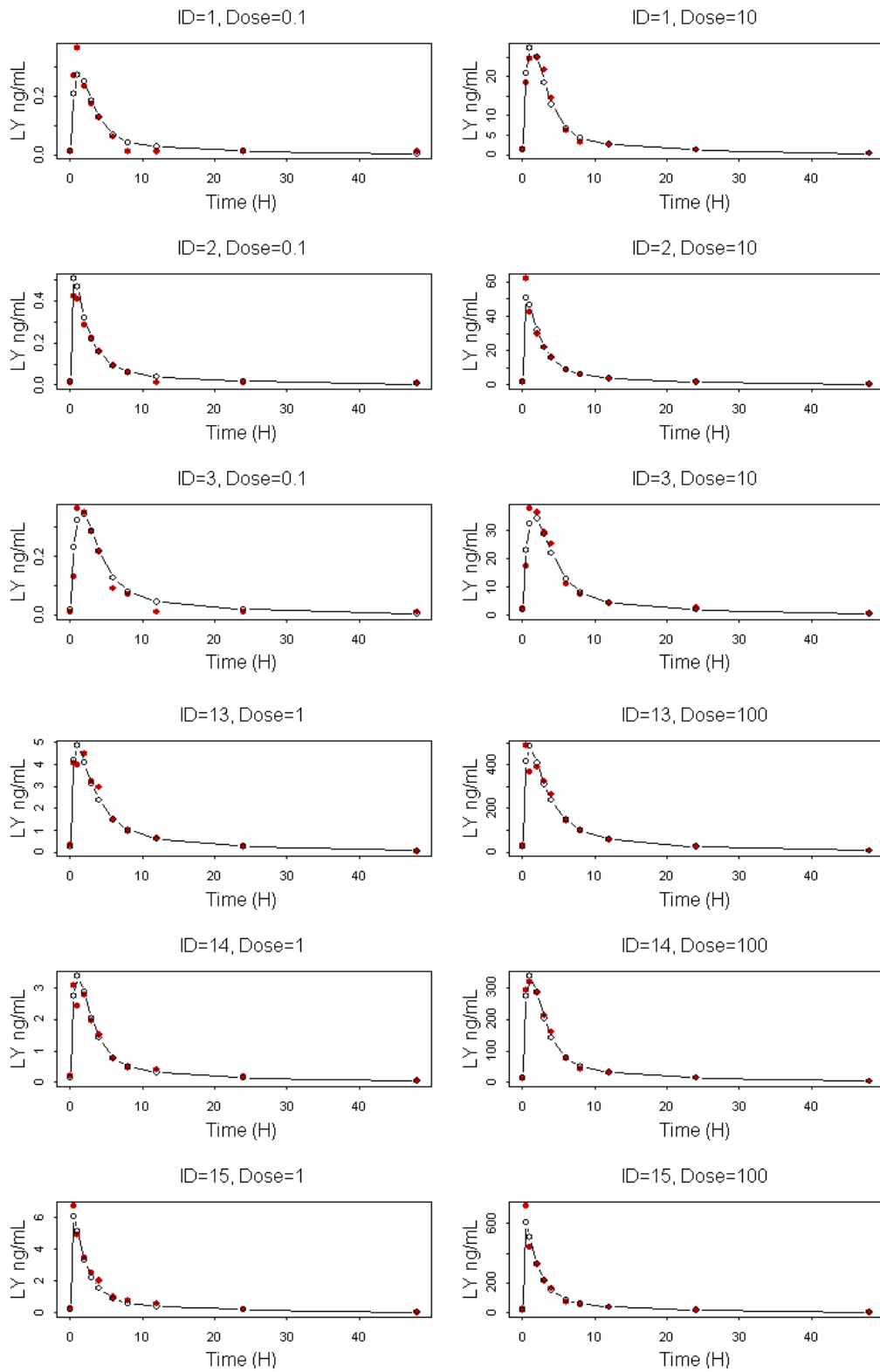
Figure S1. Pharmacokinetic Model Diagnostics: Observed and Fitted vs Time (Selected Subjects)

Model Diagnostics

Traditional model diagnostics were used to examine model fit. For all models, a plot of observed response vs. individual fitted response and a plot of weighted residuals vs. individual fitted response are provided. The identity line (ie, a line with a unit slope) and a horizontal line at zero are used as a reference, respectively. In cases of higher variability at higher response, plots of observed vs. fitted response will likely show data points resembling a wedge shape. However, when a proportional error model is used, then the more relevant, weighted residual plot should show a scatter around the zero line with constant variability for all data points.

Multivariate Response Modeling

A primary interest in the analysis of potassium and aldosterone PK/PD relationship was to assess the relative response in these 2 endpoints, and to generate simultaneous probabilistic statements about such responses. Thus, aldosterone and potassium models were fitted in pairs simultaneously (multivariate model), estimating the estimates of exposure response parameters and random variables for both responses in the same fit, along with any correlation between the parameters (multivariate variance-covariance matrix).

Model Predictions

Model predictions were calculated by applying a randomly sampled realization of model parameters from the multivariate normal distribution of the model variance-covariance matrix to the model equation, assuming a range of LY3045697 doses (up to 100 mg) or LY3045697 concentrations (up to 300 ng/mL). Performing the prediction 1000 times (at each dose/concentration level), allowed the calculation of medians (50_{th} percentile), and obtained 90% prediction intervals (5_{th} to 95_{th} percentiles) for the prediction at a given dose or concentration. In the predictions, both inter-subject variability and residual errors were ignored, and thus, the predictions represent the expected response of the typical subject. This approach is utilized because the main interest is to derive inferences about the underlying truth of drug response rather than the performance in a trial. The predictions were also used, by taking the difference between treatments for example, LY3045697 – placebo, to produce placebo subtracted LY3045697 effect or LY3045697 – SPN to produce SPN subtracted LY3045697 effect. Subsequent to taking the difference, the 90% prediction intervals were also calculated.

Software

All data preparation and pharmacokinetic analyses where performed using SAS version 9.1.3 and Splus 6.2 Professional edition. Population PK, dose-response, and PK-PD models were performed using a maximum likelihood approach to nonlinear mixed effects regression as implemented in the S-PLUS function "nlme."

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Table S2. Descriptive Statistics of Potassium Clearance by Treatment after Single Dosing

		Potassium Clearance (L/min)							
		LY3045697							
Statistic	Placebo (N=24)	0.1 mg (N=6)	0.3 mg (N=6)	1 mg (N=6)	3 mg (N=6)	10 mg (N=6)	30 mg (N=6)	100 mg (N=6)	300 mg (N=6)
n	24	6	6	6	6	6	6	6	6
mean	0.0119	0.0110	0.0130	0.0141	0.0118	0.0118	0.0108	0.0092	0.0092
SD	0.0040	0.0045	0.0024	0.0043	0.0024	0.0045	0.0019	0.0007	0.0022
CV (%)	34	40	18	31	20	38	18	7	27
median	0.0118	0.0105	0.0137	0.0130	0.0113	0.0122	0.0102	0.0091	0.0099
min	0.005	0.005	0.009	0.010	0.009	0.006	0.009	0.008	0.005
max	0.024	0.017	0.016	0.022	0.015	0.019	0.014	0.010	0.010

CV = coefficient of variance; max = maximum; min = minimum; N = number of subjects; SD = standard deviation
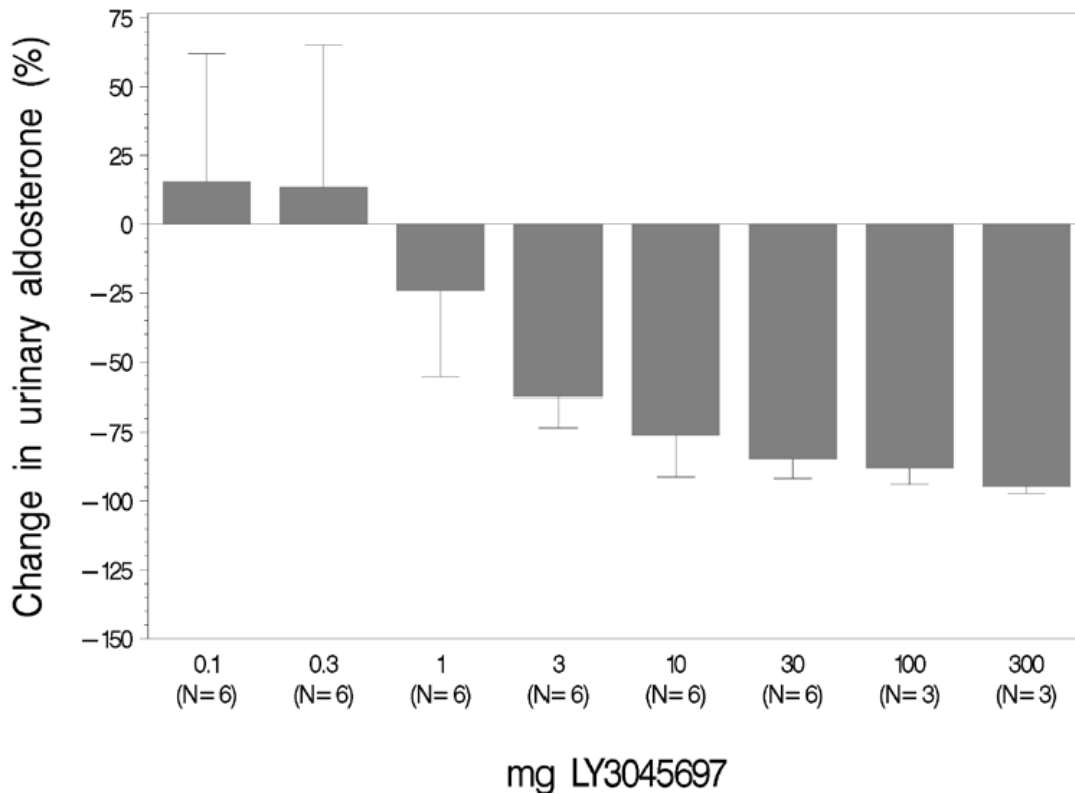

Figure S2. Change in Aldosterone Excretion in Urine After a Single Dose as a Percentage of Placebo (Urine Collected from 4 to 24 Hours Post-dose)
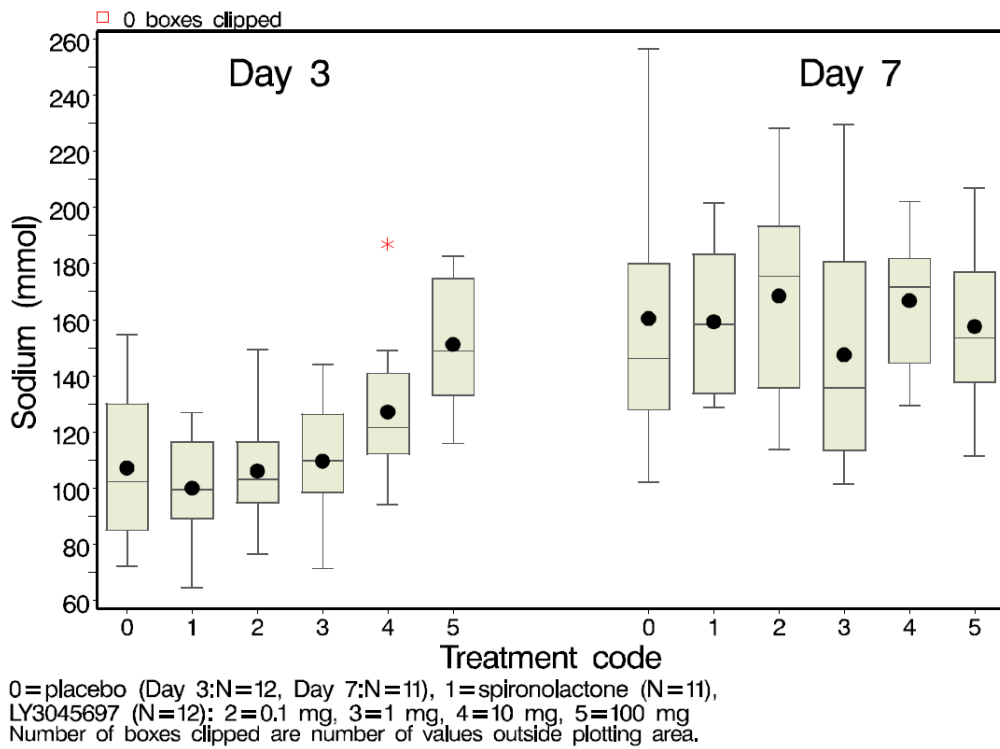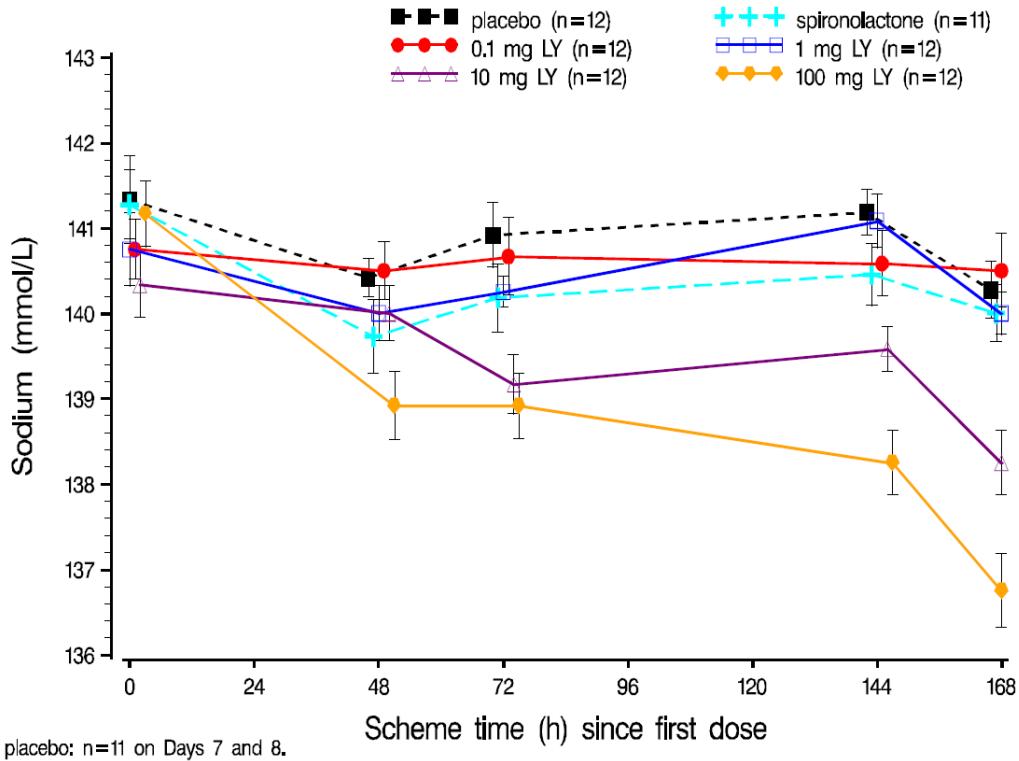
Figure S3. Mean Serum Sodium Over Time After Multiple Dosing (Top Panel) and Mean Sodium Excretion After Multiple Dosing on Day 3 and Day 7 (Bottom Panel)
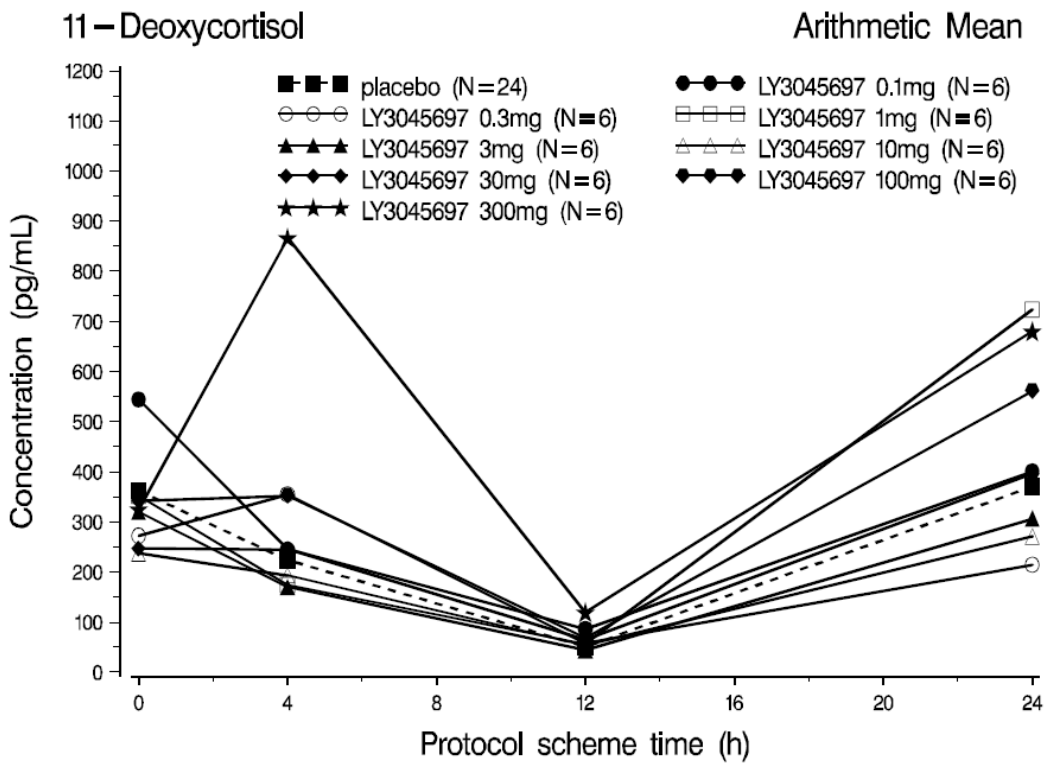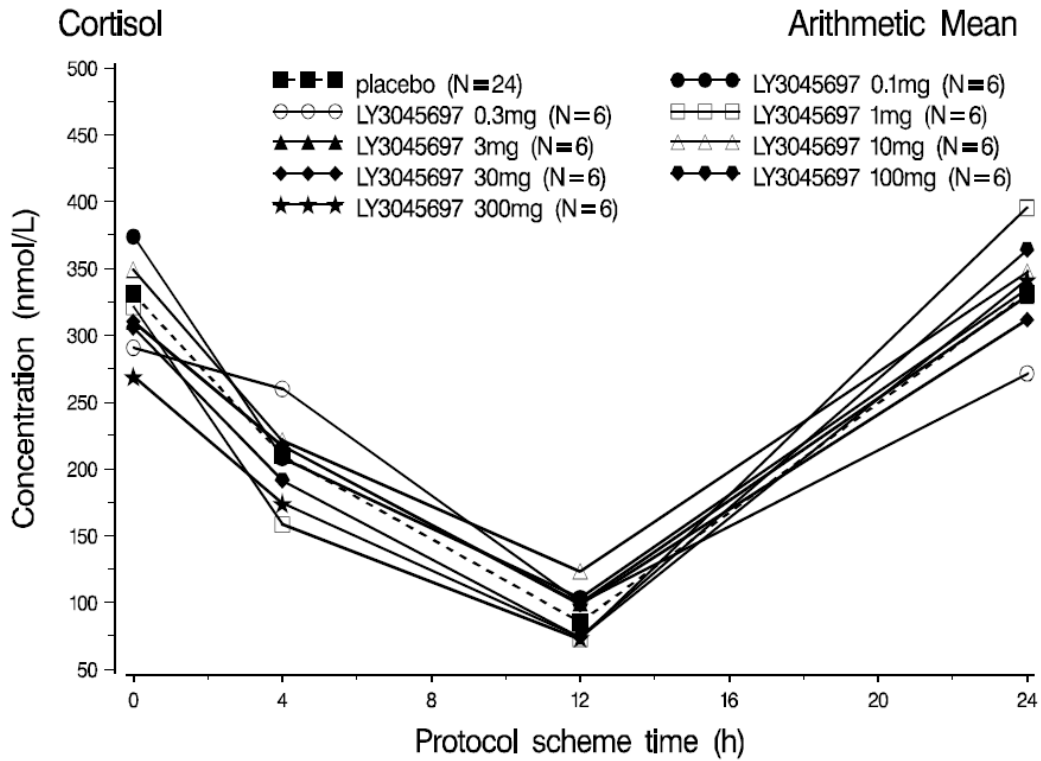
Figure S4. Mean Basal Cortisol (Top Panel) and 11-deoxycortisol (Bottom Panel) After Single Dosing

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