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ESTIMATING SYSTEMIC EXPOSURE TO ETHINYL ESTRADIOL FROM AN ORAL CONTRACEPTIVE

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Abstract

Objectives—This study was conducted to compare single-dose pharmacokinetics of ethinyl estradiol in an oral contraceptive to steady-state values, and to assess whether any simpler measures could provide an adequate proxy of the ‘gold standard’ 24-hour steady-state area-under-the-curve. Identifying a simple, less expensive, measure of systemic ethinyl estradiol exposure would be useful for larger studies designed to assess the relationship between an individual’s ethinyl estradiol exposure and her side effects.

Study Design—We conducted a 13 samples over 24 hours pharmacokinetic analysis on day 1 and day 21 of the first cycle of a monophasic oral contraceptive containing 30 mcg ethinyl estradiol and 150 mcg levonorgestrel in 17 non-obese healthy white women. We also conducted an abbreviated single dose 9-sample pharmacokinetic analysis after a month washout. Ethinyl estradiol was measured by liquid chromatography-tandem mass spectrometry. We compared results of full 13-sample steady-state pharmacokinetic analysis with results calculated using fewer samples (9 or 5) and following the single doses. We calculated Pearson correlation coefficients to evaluate the relationships between these estimates of systemic ethinyl estradiol exposure.

Results—The area-under-the-curve, maximum (C_{max}), and 24-hour (C_{24}) values were similar following the two single oral contraceptive doses (area-under-the-curve, $r = 0.92$). The steady-state 13-sample 24-hour area-under-the-curve was highly correlated with the average 9-sample

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Conflicts of Interest: C.L.W. is a paid consultant to Merck and Bayer, both of which manufacture oral contraceptives; however, not the oral contraceptive evaluated in this study. The remaining authors report no conflict of interest.

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area-under-the-curve after the two single doses ($r = 0.81$, $p = 0.0002$). This correlation remained the same if the number of samples was reduced to 4, taken at time 1, 2.5, 4 and 24 hours. The C_{24} at steady-state was highly correlated with the 24-hour steady-state area-under-the-curve ($r = 0.92$, $p < 0.0001$). The average of the C_{24} values following the two single doses was also quite highly correlated with the steady-state area-under-the-curve ($r = 0.72$, $p = 0.0026$).

Conclusions—Limited blood sampling, including results from two single doses, gave highly correlated estimates of an oral contraceptive user's steady-state ethinyl estradiol exposure.

Keywords

ethinyl estradiol; oral contraceptives; pharmacokinetics; steady-state; single-dose

Background and Objective

Oral contraceptives (OCs) are prescribed with a general approach of using the lowest effective dose. Most currently used OCs contain 20–35 mcg of ethinyl estradiol (EE2) along with one of several progestins. Venous thromboembolism (VTE) is the main reason to avoid higher EE2 doses;^{1–3} however, the lowest EE2 doses are associated with more breakthrough bleeding.⁴ These associations have been defined through studies evaluating the administered dose. The dose in each daily tablet may, however, be a poor indicator of a particular woman's systemic exposure. Numerous pharmacokinetic (PK) studies demonstrate that the steady-state (SS) levels of EE2 vary widely among women taking the same OC.^{5–7} These between-woman differences are larger than the dose differences between current and older OC formulations.

Among women taking an OC, individual-level systemic exposure to EE2 could be related to the frequency of side effects and such measures can indicate important drug interactions.^{8–9} To evaluate the possible relationships between individual systemic exposure to EE2 and OC side effects would generally require studies much larger than the typical 15–20 participants in a detailed PK study, and would be prohibitively expensive. Small intensive PK studies are necessary during drug development; however, they are not useful tools for pharmaco-epidemiological studies of drug effects. Studies of side effects and individual systemic exposure have not been done with an OC.

The objective of the study described here was to estimate systemic exposure to EE2 among a group of healthy white women using standard 13-sample PK techniques, and then to assess whether any simpler measures could provide an adequate proxy of the 'gold standard' 24-hour SS area-under-the-curve (AUC). Identifying a simple, less expensive measure of EE2 exposure would enable the development of large studies to assess the relationship between an individual's EE2 exposure and her side effects.

Materials and Methods

This single-arm, open label clinical trial took place at Columbia University Medical Center (CUMC) after Institutional Review Board approval. Participants were aged 18–35 years, self-identified as white/Caucasian, and provided written informed consent prior to enrollment. We excluded women with medical contraindications to use of combined

hormonal contraception.¹⁰ Additional exclusion criteria included: previous hysterectomy or oophorectomy; cycles > 35 days or irregular; childbirth within 6 months; breastfeeding; current smoker; body mass index ≥ 30.0 kg/m²; and use of OCs within 1 month or injectable contraception within 6 months.

After telephone screening, women attended a pretreatment visit for informed consent and full assessment of eligibility. At baseline we assessed blood pressure; height, weight; urine pregnancy test; and hemoglobin level to screen for anemia (hemoglobin < 10 mg/dL) prior to study blood draws. We asked participants to abstain from use of acetaminophen, ibuprofen, and aspirin and to avoid grapefruit juice throughout the study, alcohol within 24 hours, and caffeine within 1 hour of study visits.

The study OC contained 30 mcg EE2 and 150 mcg levonorgestrel packaged with 21 active and 7 placebo tablets (PortiaTM, Teva, Philadelphia, PA, USA). Treatment began within 7 days of the start of menses. After completing 21 active pills, participants had a 5 week, OC-free wash-out. Upon next menses, each participant returned to take a single OC tablet. A study coordinator directly observed OC intake on study visit days, and instructed participants to take each OC at the same time using daily alarms.

Participants made 10 study visits over 9 weeks. The 5 study visits of interest to the results presented here occurred on OC cycle day 1 (OC1, referred to here as single-dose 1, SD1), cycle days 2 and 3 (OC2, OC3), day 21 (OC21 or SS), and the visit for a single OC tablet around day 60 after study entry (single-dose 2, SD2). Participants underwent multiple timed venous blood collections on days 1, 21, and 60 for PK testing during which they were admitted to the Irving Institute of Clinical and Translational Research at CUMC. Using an indwelling catheter in an ante-cubital vein, 13 samples were collected at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 16, and 24 hours (t_0 , $t_{0.5}$, ... t_{24}) after OC administration on days 1 and 21. On day 60, the first 8 specimens up to 4 hours and the specimen at 24 hours were collected (9 samples); samples were also collected at 48 and 72 hours. OC administration occurred immediately after each t_0 blood draw. Samples were allowed to clot for 30 minutes at room temperature, were centrifuged at 3000 rpm at 4°C for 10 minutes and stored in 1 mL aliquots at -80°C. Levels of corticosteroid-binding globulin (CBG) were measured in serum specimens collected at t_0 on days 1, 21, and 60 to monitor treatment compliance.¹¹

Laboratory methods

EE2 concentrations were measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS). In short, EE2 was measured in serum after liquid/liquid extraction using tetra-deuterated-EE2 as the internal standard. EE2 was derivatized with dansyl chloride prior to analysis. The steroids were quantified by positive electrospray ionization in multiple reaction-monitoring mode using the Waters Xevo TQ-S system (Waters, Milford, MA, USA). The method was linear between 2.5 and 100 pg/mL (limit of quantification: 2.5 pg/mL). Intra- and inter-assay precision were <3.9% and <4.4%. The EE2 levels obtained using this assay are lower than, but highly correlated with ($r = 0.95$), those obtained using the radioimmunoassay (RIA) we have previously used among women using the same OC.^{7,12} CBG was measured using an RIA kit (IBL-America, Minneapolis, MN, USA).

Pharmacokinetic Analysis

The PK analyses were conducted using the non-compartmental analysis procedure, pkexamine, with the trapezoidal rule in the statistical package STATA12 (STATA Corporation, Austin, TX, USA). These analyses were confirmed to give results identical to those obtained with the non-compartmental analysis procedure in WinNonLin (Certara, St. Louis, MO, USA). The AUCs at SD1 and SS using all samples are noted as $AUC_{SD1, 0-24}$ and $AUC_{SS, 0-24}$. The AUCs at SD1 and SD2 ignoring any samples taken at time points > 4 hours and < 24 hours are noted as $AUC_{SD, 0-4-24}$. For SD1, the AUC from 0 to infinity, $AUC_{SD1, 0-inf}$, was calculated in the standard manner by estimating the terminal elimination rate, k_{elim} , of EE2 using the EE2 values at 12, 16, and 24 hours with a linear fit to the log EE2 values, and then using the fitted equation to 'correct' the 12, 16 and 24 hour values and extending the linear fit to infinity.

Statistical Methods

We examined results for outliers using a standard modified Z score approach with the resistant MAD estimate of the underlying variability (drastically reducing the effect of any outliers) of the statistic.¹³ Values of the modified Z score > 4 were considered outliers.¹³

The validity of the day 21 SS AUC (and other PK parameter estimates at SS) is somewhat dependent on whether the participant had taken her previous tablets very close to 24 and 48 hours prior to taking the day 21 tablet; at steady-state, the EE2 value immediately prior to taking the day 21 tablet should be roughly equal to the EE2 value 24 hours after taking the day 21 tablet. To check on this we calculated the difference between the EE2 levels at the beginning (C_0) and end (C_{24}) of day 21. We excluded women whose difference was substantial using the modified Z score approach described above.

Estimation of $AUC_{SD1, 0-inf}$ is strongly dependent on the estimation of k_{elim} . We investigated the possibility of outliers in k_{elim} by carrying out the modified Z score approach on the related half-life estimates ($half-life = \ln(2)/k_{elim}$).

Standard errors, 95% confidence intervals and p-values of the Pearson correlation coefficients (r values) were calculated based on Fisher's z transformation of r.

Results

Ninety-one women expressed interest in the study; 24 met eligibility criteria and signed an informed consent. One changed her mind and five others withdrew prior to treatment: three due to scheduling conflicts, one due to fear of needles and one due to the use of an excluded medication. Another woman withdrew during the first study visit due to poor venous access; thus, 17 women participated in the study. One participant missed the day 60 visit; the others completed all scheduled study visits.

Participants underwent the OC day 1 visit within 7 days of the start of menses (median day 6). Each participant selected a pill-taking time to maintain throughout the study and study visit times were coordinated with OC intake times; 5 chose a pill time between 9 AM-12 PM, 8 between 12-5 PM, and 4 between 5-7:30 PM; the usual pill time was unrelated to the

following PK results. Pill intake was directly observed at study visits on days 1, 2, 3, 4, 7, 21 and 60. All participants had an increase in CBG levels from baseline to day 21, and a decrease to baseline levels at day 60, consistent with good OC compliance.¹¹

Figure 1A shows the difference between the C_0 and C_{24} EE2 results on day 21 (SS). Three participants showed clear evidence that the previous tablet had been taken much more recently than 24 hours before. The estimated $AUC_{SS, 0-24}$ for these 3 women are therefore much higher than their true $AUC_{SS, 0-24}$; their results have been excluded from Tables 2–3 and Figure 2. The woman who missed her day 60 visit was one of these three. Figure 1B shows that a single participant had an unusually long elimination half-life ('outlier') following SD1; this individual's results were excluded from the calculation of half-life and $AUC_{SD1, 0-inf}$.

Table 1 shows baseline characteristics of all 17 women who underwent study treatment and sampling; the 3 women excluded due to poor compliance at SS and the 7 women who never received study treatment were similar to the other participants (data not shown). Table 2 shows PK results for the 14 timing-compliant participants. The $AUC_{SD, 0-4-24}$, C_{max} , and C_{24} values were similar following SD1 and SD2; and the correlation between $AUC_{SD1, 0-4-24}$ and $AUC_{SD2, 0-4-24}$ was high ($r = 0.92$). As expected, AUC , C_{max} , and C_{24} at steady-state were higher than the values following the single doses. The $AUC_{SD1, 0-inf}$ was lower than the $AUC_{SS, 0-24}$ in agreement with a shorter half-life and a lower change from baseline of C_{max} of SD1.

Correlations between several variations of the AUC_{SD} and the $AUC_{SS, 0-24}$ are given in Table 3A. The correlations between measures of AUC_{SD1} and AUC_{SS} were similar whether AUC_{SD1} was calculated using all 13 samples as $AUC_{SD1, 0-24}$ or $AUC_{SD1, 0-inf}$ or the subset of 9 excluding samples drawn between 4 and 24 hours, $AUC_{SD1, 0-4-24}$. The correlation with $AUC_{SS, 0-24}$ resulting from averaging the AUC_{0-4-24} values obtained after the two single doses was $r = 0.80$ ($p = 0.0002$; see Figures 2A and 2B). Using results from only 6 samples on each of these days (t_0 , t_1 , t_2 , t_3 , t_4 , and t_{24}) was equivalent to considering the results from 9 samples ($r = 0.81$). We also assessed the correlation using only t_1 , $t_{2.5}$, t_4 , and t_{24} ; these correlations were indistinguishable from those using 6 or 9 samples.

Table 3B shows the correlations between the $AUC_{SS, 0-24}$ and several trough values. C_{24} on the day of the SS assessment was highly correlated with the $AUC_{SS, 0-24}$ ($r = 0.92$, $p < 0.0001$). C_{24} on SD1 was also correlated with $AUC_{SS, 0-24}$ but the result was not statistically significant ($r = 0.44$, $p = 0.12$) and the average of the C_{24} values on SD1 and SD2 was quite highly correlated with $AUC_{SS, 0-24}$ ($r = 0.72$, $p = 0.0026$). We could not include trough values from additional days (48 and 72 hours) after the single dose at day 60 to improve these correlations, as almost half of the EE2 values at 48 hours and almost all of the values at 72 hours were below the limit of quantification of our assay.

Although the $AUC_{SD, 0-4-24}$ from SD1 and SD2 were very highly correlated, the within subject $AUC_{SD, 0-4-24}$ were quite variable. Overall, the within-subject standard deviation was 72.7 pg·h/mL, a value that was 49% of the between-subject standard deviation (148.8 pg·h/mL). It is for this reason that having more than one SD made such an improvement in

the correlation with the SS value. Most subjects' repeat values were very close; the high within-subject variability was driven by the values from 2 subjects; excluding them from the analysis reduced the estimated within-subject standard deviation to 40.8 pg·h/mL. We were not able to identify any reason for the hyper-variability of these 2 subjects, and thus did not exclude them from any of the analyses presented.

Comment

We have demonstrated that a limited number of blood samples can lead to good estimates of an OC user's systemic EE2 exposure. A single well-timed trough value at SS was highly correlated ($r = 0.92$, $p < 0.0001$) with a full 24 hour 13-sample SS assessment. Reassessment of our data from a previous PK study using the same OC confirmed this high correlation ($r = 0.94$, data not shown).⁷ Trough levels are often used to assess systemic exposure for therapeutic drug monitoring with drugs where these correlations are similar to or lower than found here for EE2. Using a single SS trough value of EE2 as a proxy for systemic exposure may be valuable for pharmaco-epidemiology studies that require a simple, low cost approach; this alternative would need only one or two samples per woman. Recruiting women into such a study ought to be far easier than recruiting for a study that requires an overnight stay. An important caveat is that any PK study (whether the full 13-sample "gold standard" or more limited sampling) needs accurately timed specimens; thus, in all PK studies it would be preferable to administer the day 20 as well as the day 21 OC tablets to the participant to avoid error. Using a single sample does have a cost in precision, and thus studies relying on this timed C_{24} measure would need to be somewhat larger than studies relying on 13-sample steady-state measurement. In order to achieve the same statistical precision and power, one would need to increase the sample size of such studies by a factor of $1/r^2$. For example, a 24 hour study requiring N participants to address a question would need to enroll $1.18 \times N$ women ($1/0.92^2 = 1.18$) to address the same question using just 1 sample.

The mean of the AUC obtained after two single doses was also highly correlated with the steady-state EE2 exposure ($r = 0.81$, $p = 0.0002$), thus permitting estimation of steady-state EE2 exposure in women without requiring a full cycle of dosing. For example, this would permit the study of EE2 exposure among women who have experienced a serious adverse event (SAE) such as VTE during past OC use, that is, among women who cannot safely undergo extended re-exposure. The approach of averaging AUC results with limited sampling after two single doses would require collecting only 8 samples per woman (as one can argue that the t_0 value in women not using any OC would be zero, and the t_0 sample would thus be unnecessary), but also would require a greater sample size - instead of N participants, it would require $1.52 \times N$ ($1/0.81^2 = 1.52$) participants. Thus, this approach would require more participants, but no increase in the total number of samples for analysis ($13 \times N$ vs $12.2 \times N$). Recruitment and study operations would be easier without requiring an overnight stay.

In a situation where the pharmacokinetics stays constant over the OC cycle, $AUC_{SD1, 0-inf}$ will be equal to $AUC_{SS, 0-24}$. This was not observed in our data. The $AUC_{SD1, 0-inf}$ was lower than the $AUC_{SS, 0-24}$, in agreement with the shorter half-life and the lower change

from baseline of $C_{\max}$ of SD1 (see Table 2). Contrary results were found in a similar study conducted by de Visser and colleagues.¹⁴ A possible reason for this discrepancy is that their RIA assay found valid values at 48 and 72 hours after a single dose, thus they used values at 24, 48 and 72 hours to estimate the elimination half-life of EE2 at single-dose and hence $AUC_{0-\infty}$. Use of these later measurements led to a greater estimate of half-life and $AUC_{0-\infty}$ than our use of EE2 values at 12, 16 and 24 hours only (done because our LC-MS/MS measures of EE2 did not give valid estimates at 48 and 72 hours. That study used a different progestin in combination with EE2, which may also contribute to differences in the EE2 PK estimates.

This study has several limitations. We studied only non-obese white women; while we expect that studies of other specific groups would yield comparable results when using parsimonious sampling, further study would be needed regarding mixed study groups that would be more typical of the U.S. population.¹⁵ We did not control participant dietary intake; thus, we may have missed an opportunity to reduce PK variability. We collected only 9 samples on SD2 and more complete sampling would have allowed additional, potentially valuable, comparisons. More broadly, a larger sample size in this study would have increased the precision of the results. Many of the EE2 levels at 48 and 72 hours after single-OC dosing were undetectable; using a more sensitive assay would have permitted incorporating these into our calculations. In addition, while both of the approaches suggested by our data have some efficiencies, they do not allow the estimation of all PK parameters. This study evaluated EE2 in combination with LNG; the correlations reported here may need to be investigated for other OC formulations before more widespread use. We also need similar evaluations of LNG and other contraceptive progestins. Additionally, these results may not be applicable to studies using other laboratories unless the assays used have been validated against the assays used here, or unless the comparisons reported here are replicated.

For many drugs the therapeutic window is narrow, and adverse events increase with greater systemic exposure. To our knowledge, no studies have evaluated the risk of OC side effects according to a user's individually measured systemic exposure. Clinical experience indicates that many patients believe that side effects are related to dose; however, we lack empirical evidence for this belief, particularly at the individual level. Using conventional SS 24 hour PK assessments is not a practical way to explore such hypotheses. Moreover, to investigate the relationship between systemic exposure and SAE in women at high risk of an adverse event, one must limit the systemic exposure to the drug to an absolute minimum. The results presented here show that studies of the association between side effects and individual level exposure are possible by use of very restricted sampling of EE2 values.

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Clinical Implications

- Pharmacokinetic studies can define women's systemic exposure to ethinyl estradiol in oral contraceptives.
- Our study indicates that a single precisely timed trough sample at steady-state is highly correlated with estimates from a full pharmacokinetic study.
- Pharmacokinetic estimates of the area-under-the-curve following a single-dose exposure are also highly correlated with estimates at steady-state.
- These results may be useful for planning pharmaco-epidemiologic studies to assess ethinyl estradiol exposure and adverse events.

Figure 1. Identification of data outliers

1A. EE2 concentration at time 0 minus concentration at 24 hours following OC ingestion on cycle day 21, n = 17.

1B. Elimination half-life calculated based on serum levels following a single OC dose, n = 17.

Figure 2. Correlations between measures of AUC_{SD} and AUC_{SS}

2A. Single-dose 1 AUC_{0-inf} versus steady-state AUC_{0-24}

2B. Average of single-doses 1 and 2 $AUC_{0-4,24}$ versus steady-state AUC_{0-24}

Table 1

Baseline characteristics of study participants (n = 17)

Variable	Study Participants
Age	24.9 (± 3.9)
Height (cm)	168.1 (± 7.7)
Weight (kg)	64.0 (± 10.6)
BMI (kg/m ²)	22.6 (± 3.1)
Ever been pregnant	2 (11.8)
Ever given birth	0 (0.0)
Previously used OCPs	11 (64.7)

Values are shown as mean ($\pm$ SD) or n (%).

Table 2

OC pharmacokinetic parameter estimates of single-dose versus steady-state administration (n=14)

Parameter	Single Dose 1	Single Dose 2 [*]	Steady State ^a
AUC ₀₋₂₄ ^b (pg·h/mL)	322.9 (247.1, 422.0)	-	646.5 (496.3, 842.3)
AUC _{0-inf} ^c (pg·h/mL)	470.8 ^{**} (315.8, 701.9)	-	-
AUC ₀₋₄₋₂₄ ^d (pg·h/mL)	395.2 (299.7, 521.1)	384.8 (253.3, 584.6)	746.4 (567.5, 981.8)
C _{max} (pg/mL)	33.5 (21.2, 52.9)	31.2 (18.1, 53.9)	56.8 (37.9, 85.1)
t _{max} (h)	2.75 (1.5, 3.75)	2.25 (1.5, 3.75)	2.25 (1.5, 2.5)
Elimination half-life ^e (h)	14.5 ^{**} (8.9, 23.6)	-	24.0 ^{**} (16.4, 35.2)
C ₂₄ ^f (pg/mL at t=24)	6.3 (3.8, 10.5)	7.4 (4.7, 11.6)	15.9 (11.4, 22.3)

Parameter estimates shown as geometric mean±SD, except T_{max} shown as median (Q1–Q3).^a Steady-state defined at day 21 of cycle 1.^b Area under the curve (AUC) calculated from t₀ to t₂₄ using 13 measurements.^c AUC calculated from t₀ to t_{inf} using 13 measurements.^d AUC calculated from t₀ to t₂₄ using 9 measurements.^e Elimination half-life calculated using measurements from t₁₂, t₁₆, and t₂₄.^{*} AUC and elimination half-life not calculated for Single Dose 2 due to no data for t₆, t₈, t₁₂, and t₁₆.^{**} Elimination half-life and AUC_{0-inf} calculated for n=13.

Table 3Correlation between estimates of several PK parameters and steady-state AUC₀₋₂₄

A. AUC parameters	n	k	r (±95% CI)*	p-value
Steady-state AUC ₀₋₂₄	14	9	0.94 (0.82, 0.98)	<0.0001
Steady-state AUC ₀₋₄₋₂₄	14	6	0.93 (0.79, 0.98)	<0.0001
Single-dose 1 AUC _{0-inf}	13	13	0.72 (0.28, 0.91)	0.0041
Single-dose 1 AUC ₀₋₂₄	14	13	0.67 (0.22, 0.89)	0.0072
Single-dose 1 AUC ₀₋₄₋₂₄	14	9	0.74 (0.34, 0.91)	0.0016
Single-doses 1 & 2** AUC ₀₋₄₋₂₄	14	18 = 2 × 9	0.80 (0.49, 0.94)	0.0002
Single-dose 1 AUC ₀₋₄₋₂₄	14	6	0.73 (0.33, 0.91)	0.0021
Single-doses 1 & 2** AUC ₀₋₄₋₂₄	14	12 = 2 × 6	0.81 (0.49, 0.94)	0.0002
Single-dose 1 AUC ₀₋₄₋₂₄	14	4	0.73 (0.33, 0.91)	0.0021
Single-doses 1 & 2** AUC ₀₋₄₋₂₄	14	8 = 2 × 4	0.82 (0.51, 0.94)	0.0001

B. C ₂₄ parameters	n	k	r (±95% CI)*	p-value
Steady-state C ₂₄	14	1	0.92 (0.76, 0.97)	<0.0001
Single-dose 1 C ₂₄	14	1	0.44 (-0.12, 0.79)	0.1173
Single-doses 1 & 2** C ₂₄	14	2 = 2 × 1	0.72 (0.31, 0.90)	0.0026

C₂₄ at t=24.

n = number of participants used in calculation

k = number of EE2 measures used per participant

* r, Pearson correlation coefficient; CI, confidence interval calculated using Fisher's z transformation

** Mean of estimated parameters

Appendix Table 1

Regression equations describing the relationship between estimates of several PK parameters and steady-state AUC_{0-24}

PK parameters	Regression equation*
Steady-state AUC_{0-24} (k=9)	$y = 0.84x + 21.5$
Steady-state AUC_{0-24} (k=6)	$y = 0.85x + 13.8$
Single-dose 1 AUC_{0-inf}	$y = 0.62x + 365.6$
Single-dose 1 AUC_{0-24}	$y = 1.30x + 233.1$
Single-dose 1 AUC_{0-24} (k=9)	$y = 1.14x + 201.1$
Single-doses 1 & 2** AUC_{0-24} (k=9)	$y = 0.94x + 278.6$
Single-dose 1 AUC_{0-24} (k=6)	$y = 1.17x + 189.6$
Single-doses 1 & 2** AUC_{0-24} (k=6)	$y = 0.97x + 266.4$
Single-dose 1 AUC_{0-24} (k=4)	$y = 1.12x + 210.1$
Single-doses 1 & 2 AUC_{0-24} (k=4)	$y = 0.76x + 281.4$
Steady-state C_{24}	$y = 32.65x + 121.9$
Single-dose 1 C_{24}	$y = 24.21x + 497.6$
Single-doses 1 & 2** C_{24}	$y = 45.24x + 325.5$

* x, PK parameter; y, gold standard 13-sample steady-state AUC_{0-24}

** Mean of estimated parameters