Original Manuscript

Comparison of Steroidogenic Exposure Following the Administration of Repository Corticotropin Injection With a Synthetic $ACTH_{1-24}$ Depot and Methylprednisolone in Healthy Subjects

Clinical Pharmacology in Drug Development
2021, 10(7) 777–788

DOI: 10.1002/cpdd.894

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Abstract

The pharmacokinetics (PK) and pharmacodynamics (PD) of clinically relevant doses of repository corticotropin injection (Acthar Gel) and synthetic $ACTH_{1-24}$ depot have not been fully characterized. We compared the steroidogenic exposure of repository corticotropin injection and synthetic $ACTH_{1-24}$ depot in healthy adults at therapeutic doses using data from 2 separate phase 1 studies. Subjects were randomly assigned to repository corticotropin injection 40 or 80 IU subcutaneously twice weekly or 80 IU subcutaneously 3 times weekly for 15 days or to daily synthetic $ACTH_{1-24}$ depot doses of 0.5 mg subcutaneously, 0.75 mg subcutaneously, 1 mg subcutaneously, or 1 mg intramuscularly for 5 days. A population PK/PD model was developed to simulate the free cortisol exposure of a clinically relevant dose of synthetic $ACTH_{1-24}$ depot (1 mg subcutaneously twice weekly). Study drug doses were converted to methylprednisolone-equivalent doses using the steroidogenic exposure of methylprednisolone 16 mg daily as a conversion factor. Doses were also converted to prednisone equivalents using a coefficient of 1.25. These analyses revealed that the steroidogenic exposure of repository corticotropin injection at clinically relevant doses was substantially lower than that for synthetic $ACTH_{1-24}$ depot. The 3 repository corticotropin injection regimens were equivalent to approximately 5, 8, and 16 mg of daily prednisone, respectively. On the basis of simulated free cortisol exposure, synthetic $ACTH_{1-24}$ depot 1 mg subcutaneously twice weekly was comparable to 57 mg of daily prednisone. These results suggest that repository corticotropin injection has pharmacological effects that cannot be considered identical to synthetic $ACTH_{1-24}$ depot.

Keywords

repository corticotropin injection, Acthar Gel, synthetic $ACTH_{1-24}$ depot, glucocorticoids, melanocortin receptors, steroidogenic exposure, pharmacokinetics, pharmacodynamics, adrenocorticotropic hormone

Adrenocorticotropic hormone (ACTH) represents a class of medications containing natural or synthetic ACTH and/or its derivatives. Within this class, repository corticotropin injection (Acthar Gel) is the naturally sourced complex mixture of modified porcine ACTH and other related peptide analogues. The mixture is solubilized in 16% gelatin, yielding a prolonged-release formulation. A major component in the formulated complex mixture is N-25 deamidated (N25D) porcine $ACTH_{1-39}$. Repository corticotropin injection is US Food and Drug Administration (FDA)-approved for multiple indications and has demonstrated efficacy for inflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus, and multiple sclerosis.[1–6] Historically, the anti-inflammatory effects of repository corticotropin

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Submitted for publication 3 September 2020; accepted 23 November 2020.

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Manuscript submitted on behalf of the authors by MedLogix Communications, LLC (MNK@medlogix.org).

injection were thought to be attributed to its binding to melanocortin receptor (MCR) 2 in the adrenal cortex, which resulted in stimulation of endogenous cortisol.[7] However, results of nonclinical studies suggest that repository corticotropin injection also engages MCRs 1, 3, 4, and 5, which are expressed on immune cells.[8–11] Among other cellular functions, these MCRs play a key role in regulating inflammation[12]; thus, the functional activity of repository corticotropin injection at these receptors may result in a direct immunomodulatory effect that is independent of its effects on endogenous cortisol production.

Previous literature has shown that different ACTH analogues have variable binding affinities for each MCR subtype.[8,13,14] Consequently, different ACTH analogues may exhibit distinct pharmacodynamic (PD) properties. For example, MNK-1411 (synthetic $ACTH_{1-24}$ depot; Figure S1) is a prolonged-release suspension that contains the first 24 amino acids of full-length $ACTH_{1-39}$ complexed with zinc. Nonclinical studies have shown that synthetic $ACTH_{1-24}$ depot has higher functional activity at MC2R than does repository corticotropin injection.[8] In single-dose studies in rats, synthetic $ACTH_{1-24}$ depot has been shown to induce higher levels of endogenous cortisol than does repository corticotropin injection.[8]

Long-term exposure to high levels of endogenous cortisol or high doses of exogenous corticosteroids may yield undesirable side effects.[15] Potential side effects of chronic exposure include osteoporosis, hypertension, fluid retention, hypokalemia, petechiae and ecchymoses, weight gain, manifestations of latent diabetes mellitus, muscle weakness, cataracts, glaucoma, and the development of a Cushingoid state. A growing body of guidelines and expert consensus panels have recommended limiting the clinical use of exogenous corticosteroids to the lowest dose and shortest course of therapy required for treatment and exercising caution in patients who have preexisting conditions that could be exacerbated by corticosteroids (eg, systemic sclerosis, nonspecific ulcerative colitis, and ocular herpes simplex).[16]

Repository corticotropin injection is an efficacious alternative for the treatment of inflammatory conditions that have not responded to corticosteroid treatment.[1–6] However, the steroidogenic exposures of repository corticotropin injection and synthetic $ACTH_{1-24}$ at their therapeutic doses have not been directly compared. In addition, the pharmacokinetics (PK) and PD after administration of multiple doses of repository corticotropin injection and synthetic $ACTH_{1-24}$ depot have not been fully characterized in humans.

The objectives of these analyses were to present the PK of repository corticotropin injection and synthetic $ACTH_{1-24}$ depot and to compare their steroidogenic exposures at clinically relevant doses in healthy adults.

Methods

Study protocols and informed consent forms were reviewed and approved by IntegReview Institutional Review Board (Austin, Texas). All subjects gave written informed consent prior to initiation of study procedures. Research was carried out in compliance with applicable US FDA regulations and the International Council on Harmonization Good Clinical Practice guidelines and in accordance with the ethical principles that have their origins in the Declaration of Helsinki.

Cortisol data and drug concentrations for the current analyses were obtained from 2 separate phase 1 studies: one study was with repository corticotropin injection and methylprednisolone, and the other study was with synthetic $ACTH_{1-24}$ depot.

Phase 1 Study of Repository Corticotropin Injection and Methylprednisolone

This was a single-center, open-label, multiple-dose, parallel-group study in healthy male or nonpregnant, nonlactating female subjects aged 18-50 years with body mass index (BMI) $\geq$ 18.5 and $\leq$ 30 kg/m^2 and weight of 51-99 kg. The study was conducted between July 9, 2016, and September 9, 2016. All female subjects who were biologically capable of having children committed to using a double-barrier, nonhormonal method of contraception from screening through the end of study participation. Subjects were randomized to receive repository corticotropin injection (40 IU subcutaneously twice weekly or 80 IU subcutaneously 3 times weekly for 15 days) or oral methylprednisolone (16 mg once daily for 15 days followed by a tapering regimen of 8 mg once daily for 2 days and then 4 mg once daily for 2 days). The methylprednisolone cohort was included to derive steroidogenic equivalence of repository corticotropin injection. Subjects were confined to the study site (Pharmaceutical Product Development, LLC, Austin, Texas) from 2 days prior to the first dose of the study drug until day 17 or day 18 if given repository corticotropin injection and day 19 if given methylprednisolone.

Blood Samples for PK/PD Assessment. Blood samples for the assessment of N25D porcine $ACTH_{1-39}$ were collected predose and 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours after dosing on day 1 and day 15. Additional blood samples were collected predose and after breakfast on day 4, day 8, and day 11 for determination of trough concentrations of N25D porcine $ACTH_{1-39}$. For cortisol assays, blood samples were collected on day –1, day 1, and day 15 after breakfast (predose) and

0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours postdose. Following the last dose on day 15, blood samples for PK analyses were collected 36, 48, and 72 hours postdose. For methylprednisolone PK and cortisol assessment, blood samples were collected on day 1 and day 15 at the following times: predose and 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours postdose. Blood samples for trough measurements of methylprednisolone were collected on day 4, day 8, and day 11. In addition, blood samples for baseline cortisol assessment were collected at the same sampling times on day –1 as those on day 1 and day 15.

Phase 1 Study of Synthetic ACTH$_{1\text{-}24}$ Depot

This was a single-center, open-label, single-/multiple-dose study in healthy male and nonpregnant, nonlactating female subjects aged 18-55 years with BMI $\geq$ 18.5 and $\leq$ 30 kg/m^2 and weight of 51-99 kg. The study was conducted between August 24, 2016, and December 8, 2016. All female subjects who were biologically capable of having children committed to using a double-barrier, nonhormonal method of contraception from screening through the end of study participation. Subjects were assigned sequentially to 1 of 5 treatments for synthetic ACTH$_{1\text{-}24}$ depot: 0.5 mg subcutaneously once daily, 0.75 mg subcutaneously once daily, 1 mg subcutaneously once daily, and 1 mg intramuscularly once daily for 5 days or a single dose of 1.5 mg subcutaneously. The 1.5-mg subcutaneous single-dose cohort was not relevant to the current analyses, as steady-state PK/PD data were not available; therefore, this cohort was excluded from the analyses described here. Subjects were confined to the clinical pharmacology unit at the study site (Pharmaceutical Product Development, LLC, Austin, Texas) from 2 days prior to the first dose of synthetic ACTH$_{1\text{-}24}$ depot until 48 hours after the final dose of 0.5 mg subcutaneously or 1 mg subcutaneously and until 9 days after the final dose of 0.75 mg subcutaneously or 1 mg intramuscularly.

Blood Samples for PK/PD Assessment. Blood samples were collected for determination of plasma concentrations of ACTH$_{1\text{-}24}$ and of serum total and plasma-free concentrations of cortisol. Blood samples for PK were collected predose and 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, and 16 hours after dosing on study day 1 and day 5. Blood samples for trough measurements were collected on day 3 and day 4 prior to dosing. Blood samples for total and free cortisol assessment were collected on day –1, day 1, and day 5 at the following times: predose and 1, 2, 3, 4, 6, 8, 10, and 12 hours postdose. Blood samples for trough measurements were also collected on day 2, day 3, and day 4. Additional blood samples were taken 16, 18, 24, 36, and 48 hours post–day 5 dosing.

Bioanalytical Methods for Both Studies

Bioanalytical work to determine N25D porcine ACTH$_{1\text{-}39}$ concentrations in plasma samples was performed at QPS Netherlands B.V., Groningen, Netherlands. Bioanalytical work to determine the ACTH$_{1\text{-}24}$, total and free cortisol, and total and free methylprednisolone concentrations was performed at Syneos Health (formerly inVentiv Health), Quebec City, Quebec, Canada. All bioanalytical work was performed in compliance with the standard operating procedures required by each bioanalytical laboratory. Analysis of all samples followed the principles of good laboratory practice. The samples were analyzed using methods that were validated according to the FDA Bioanalytical Method Validation Guidance for Industry (May 2010). Details for these bioanalytical methods can be found in the Supplemental Material.

Steroidogenic Exposure Derivation for Methylprednisolone

Methylprednisolone is a synthetic corticosteroid with systemic action similar to but 5 times more potent than cortisol itself.[15] In addition, methylprednisolone suppresses the production of endogenous cortisol. Therefore, the free steroidogenic exposure at each sampling time produced by methylprednisolone 16 mg once daily at steady state, C_{SGfree}, was derived by:

$$C_{SGfree} = 5 \times \text{Free Conc}_{MPD\_ss} + \text{Free cortisol}_{ss} \quad (1)$$

where Free Conc_{MPD_ss} is the free drug concentration of methylprednisolone at steady state, and Free cortisol_{ss} is the free cortisol level at steady state. The area under the curve of steroidogenic exposure with time within 1 dosing interval at steady state ($AUEC_{\tau SG}$) was derived by integrating each term within 1 dosing interval in equation 1, which yielded equation 2:

$$AUEC_{\tau SG} = 5 \times AUC_{\tau} + AUEC_{\tau} \quad (2)$$

where AUC_{τ} is the area under the curve of the time-drug concentration of methylprednisolone within 1 dosing interval, and $AUEC_{\tau}$ is the area under the curve of the time course of free cortisol within 1 dosing interval at steady state.

Population PK/PD Modeling to Simulate Cortisol Levels at Clinically Relevant Doses of Synthetic ACTH$_{1\text{-}24}$ Depot

The dosing regimens of repository corticotropin injection used in this study are clinically relevant and are typically employed for the treatment of nephrotic syndrome.[17] However, the synthetic ACTH$_{1\text{-}24}$ depot study was a multiple-ascending-dose (MAD) study to characterize the PK and tolerability of synthetic

$ACTH_{1\text{-}24}$ depot; therefore, studied doses were not clinically relevant. To compare the steroidogenic exposure between repository corticotropin injection and synthetic $ACTH_{1\text{-}24}$ depot at their therapeutic doses, a model-based simulation for free cortisol exposure at 1 mg subcutaneously twice weekly of synthetic $ACTH_{1\text{-}24}$ depot was conducted. The PK and cortisol data from the MAD study were used to develop the population PK/PD model for the simulation. The 1-mg subcutaneous twice-weekly dose was selected according to the typical maintenance dose of a similar product (Synacthen Depot) that is used outside the United States for the treatment of nephrotic syndrome.[18]

The population PK/PD model for simulation was developed using the data from the phase 1 study of synthetic $ACTH_{1\text{-}24}$ depot. A 1-compartment model with a fast zero-order absorption without lag time and a slow first-order absorption with lag time resulted in the best fitting of $ACTH_{1\text{-}24}$ concentration profiles. As free plasma cortisol is responsible for the pharmacological effect, the relationship between concentrations of $ACTH_{1\text{-}24}$ and free cortisol was assessed. An indirect stimulation PK/PD model characterized the induction of free cortisol well, as given in equation 3:

$$\frac{\text{dfree cortisol levels}}{\text{dt}} = K_{in} \times \left(1 + \left[\frac{E_{max} \times C^{\gamma}(t)}{EC_{50}^{\gamma} + C^{\gamma}(t)}\right]\right) - K_{out} \times \text{free cortisol levels} \quad (3)$$

where K_{in} is the rate of secretion of free plasma cortisol, K_{out} is the first-order degradation rate constant of free plasma cortisol, C(t) is the simulated concentration of $ACTH_{1\text{-}24}$ at time t, E_{max} is the maximum effect of $ACTH_{1\text{-}24}$, EC_{50} is the concentration of $ACTH_{1\text{-}24}$ to induce 50% of maximum free cortisol, and γ is the Hill coefficient. At baseline, $K_{in} = K_{out} \times \text{Baseline\_cortisol}$. If the baseline-adjusted free cortisol level is used as a dependent variable, equation 3 can be expressed as equation 4:

$$\frac{\text{d}\Delta\text{free cortisol levels}}{\text{dt}} = K_{in} \times \left[\frac{E_{max} \times C^{\gamma}(t)}{EC_{50}^{\gamma} + C^{\gamma}(t)}\right] - K_{out} \times \Delta\text{free} \quad (4)$$

where Δfree corresponds to the baseline-adjusted free cortisol levels. The $ACTH_{1\text{-}24}$ concentration that stimulates cortisol secretion at each time was predicted using the population PK model with individual posterior Bayesian estimates.

The population PK/PD model was then used to simulate $ACTH_{1\text{-}24}$ concentrations and baseline-adjusted free cortisol levels by assuming that subjects who were included in the phase 1 synthetic $ACTH_{1\text{-}24}$ depot study were administered the clinically relevant dose mentioned above. A total of 560 replicate concentration profiles were generated.

PK and PD Parameter Derivation

PK parameters, including peak drug concentration (C_{max}), area under the concentration-time curve from time 0 to the last quantifiable observation (AUC_{last}), and AUC_{τ} (τ is 1 dosing interval) after multiple doses, were derived using noncompartmental analysis implemented in Phoenix WinNonlin (version 6.2 or higher).

PD parameters were derived using the drug model in Phoenix WinNonlin. To derive baseline-adjusted (BA) PD parameters such as maximum observed effect (E_{max}[BA]) and area under the effect time curve from 0 to the last quantifiable observation ($AUEC_{last}$[BA], or $AUEC_{\tau}$[BA]), baseline measurements (day –1) were subtracted from the corresponding times for the cortisol or steroidogenic levels after treatment with each study drug. The PD parameters of synthetic $ACTH_{1\text{-}24}$ depot at 1 mg subcutaneously twice weekly were derived using the model-simulated free cortisol data.

Methylprednisolone and Prednisone Dose Equivalence

Comparison of the steroidogenic exposure of repository corticotropin injection (40 IU twice weekly, 80 IU twice weekly, and 80 IU 3 times weekly) or synthetic $ACTH_{1\text{-}24}$ depot (1 mg subcutaneously twice weekly) was performed by converting their respective therapeutic doses to methylprednisolone or prednisone-equivalent doses, respectively. Dose conversion was calculated using baseline-adjusted free steroidogenic exposure at steady state, as given in equation 5:

$$\text{Methylprednisolone equivalent dose} = AUEC_{ss\_week}/AUEC_{\tau SG} \times 16\ \text{mg} \quad (5)$$

where $AUEC_{ss\_week}$ is the free cortisol exposure within a week following either repository corticotropin injection or synthetic $ACTH_{1\text{-}24}$ depot administration, and $AUEC_{\tau SG}$ is the daily free steroidogenic exposure at 16 mg once daily of methylprednisolone. Steroidogenic exposure by methylprednisolone was assumed to be dose-proportional (see Discussion). The equivalent prednisone dose was calculated from the methylprednisolone dose using a coefficient of 1.25.[15]

Results

Subject Disposition

Forty-eight healthy subjects were enrolled in the study evaluating repository corticotropin injection and

Table 1. Demographic and Baseline Characteristics of Subjects Receiving Repository Corticotropin Injection, Synthetic $ACTH_{1-24}$ Depot, and Methylprednisolone[a]

	Repository Corticotropin Injection			Synthetic $ACTH_{1-24}$ Depot				Methylprednisolone
Variable	40 IU SC BIW (n = 12)	80 IU SC BIW (n = 12)	80 IU SC TIW (n = 12)	0.5 mg SC QD (n = 14)	0.75 mg SC QD (n = 14)	1 mg SC QD (n = 14)	1 mg IM QD (n = 13)	16 mg PO QD (n = 12)
Age (y), mean (SD)	34 (9)	32 (8)	32 (7)	33 (11)	35 (9)	29 (10)	33 (10)	32 (7)
Male, n (%)	8 (67)	9 (75)	9 (75)	11 (79)	8 (57)	8 (57)	7 (54)	9 (75)
Race, n (%)								
White	1 (8)	5 (42)	8 (67)	10 (71)	10 (71)	9 (64)	8 (62)	6 (50)
Black	9 (75)	7 (58)	4 (33)	4 (29)	4 (29)	4 (29)	5 (38)	3 (25)
Asian	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (7)	0 (0)	1 (8)
American Indian or Alaska Native	1 (8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Other	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (17)
Multiracial	1 (8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	10 (83)
Ethnicity, n (%)								
Hispanic or Latino	4 (33)	2 (17)	3 (25)	6 (43)	4 (29)	6 (43)	3 (23)	2 (17)
Weight (kg), mean (SD)	76 (11)	83 (11)	71 (8)	79 (13)	78 (10)	74 (11)	74 (12)	75 (11)
BMI (kg/m^2), mean (SD)	25 (2)	26 (2)	24 (2)	26 (3)	26 (2)	25 (3)	24 (2)	24 (4)

$ACTH_{1-24}$, first 24 amino acids of adrenocorticotropic hormone; BIW, twice weekly; BMI, body mass index; IM, intramuscularly; PO, orally; QD, daily; SC, subcutaneously; SD, standard deviation; TIW, 3 times weekly.
[a]All subjects who received at least 1 dose of study drug.

methylprednisolone. All subjects were included in the PK and PD analyses. One subject withdrew from the repository corticotropin injection 80-IU subcutaneous twice-weekly group before dosing on day 15 because of the presence of ventricular extrasystoles on an electrocardiogram. The subject was asymptomatic, and the event was considered mild. Sixty-nine healthy subjects were enrolled in the study evaluating synthetic $ACTH_{1-24}$ depot; 14 subjects were included in the 1.5-mg subcutaneous single-dose cohort, which was excluded because of the absence of steady state PK/PD data. Fifty-five subjects were included in the PK and PD analyses described here, and all subjects completed the study.

Demographics and baseline characteristics for treatment groups across both studies were comparable (Table 1). Most subjects were white (55%), male (67%), and of non-Hispanic or Latino ethnicity (71%). Mean ages ranged from 29 to 35 years.

PK Parameters

PK parameters were derived for subjects receiving repository corticotropin injection or synthetic $ACTH_{1-24}$ depot. A major component in the formulated repository corticotropin injection complex mixture is N25D porcine $ACTH_{1-39}$, which was used as the PK marker. C_{max} and AUC_{0-24} for N25D porcine $ACTH_{1-39}$ and synthetic $ACTH_{1-24}$ depot are presented in Tables 2 and 3, respectively. Plasma concentrations of N25D porcine $ACTH_{1-39}$ and $ACTH_{1-24}$ fell below the quantification limit before 24 hours postdosing. Therefore, no accumulation was observed in drug exposure for repository corticotropin injection (as measured by N25D porcine $ACTH_{1-39}$) with either twice-weekly or 3-times-weekly dosing. However, with multiple dosing of repository corticotropin injection, AUC_{last} on day 15 appeared to be higher than that of day 1 for each dose group. In contrast, with multiple dosing of synthetic $ACTH_{1-24}$ depot, AUC_{last} on day 5 appeared to be lower than that of day 1.

PD Parameters Derived From Observed Cortisol Levels

E_{max}[BA] and $AUEC_{24}$[BA], derived from baseline-adjusted cortisol values from the administration of repository corticotropin injection and synthetic $ACTH_{1-24}$ depot, are presented in Table 4. Total or free cortisol exposure following the administration of

Table 2. Mean (SD) PK Parameters for N25D Porcine $ACTH_{1-39}$[a] After Administration of Repository Corticotropin Injection[b]

	40 IU SC BIW		80 IU SC BIW		80 IU SC TIW	
	Day 1 (n = 12)	Day 15 (n = 12)	Day 1 (n = 12)	Day 15 (n = 11)	Day 1 (n = 12)	Day 15 (n = 12)
C_{max} (pg/mL)	134 (59)	146 (85)	225 (121)	228 (103)	375 (388)	301 (267)
AUC_{last} (pg·h/mL)	305 (191)	415 (295)	664 (473)	643 (338)	768 (535)	856 (507)

$ACTH_{1-39}$, the 39 amino acids of adrenocorticotropic hormone; AUC_{last}, area under the concentration-time curve from time 0 to the time of last quantifiable concentration; BIW, twice weekly; C_{max}, peak drug concentration; N25D, N-25 deamidated; PK, pharmacokinetic; SC, subcutaneously; SD, standard deviation; TIW, 3 times weekly.

[a]A major component of the formulated repository corticotropin injection complex mixture is N25D porcine $ACTH_{1-39}$, which was used as the PK marker.

[b]All subjects whose pharmacokinetic profile contained at least 4 consecutive data points with quantifiable concentrations.

Table 3. Mean (SD) PK Parameters After Administration of Synthetic $ACTH_{1-24}$ Depot[a]

	0.5 mg SC QD		0.75 mg SC QD		1 mg SC QD		1 mg IM QD	
	Day 1 (n = 14)	Day 5 (n = 14)	Day 1 (n = 14)	Day 5 (n = 14)	Day 1 (n = 14)	Day 5 (n = 14)	Day 1 (n = 13)	Day 5 (n = 13)
C_{max} (pg/mL)	121 (69)	89 (34)	225 (133)	165 (104)	237 (119)	196 (90)	257 (90)	213 (66)
AUC_{last} (pg·h/mL)	224 (145)	133 (58)	426 (342)	422 (318)	517 (217)	454 (256)	646 (341)	490 (244)

$ACTH_{1-24}$, the first 24 amino acids of adrenocorticotropic hormone; AUC_{last}, area under the concentration-time curve from time 0 to the time of last quantifiable concentration; C_{max}, peak drug concentration; IM, intramuscularly; PK, pharmacokinetic; QD, daily; SC, subcutaneously; SD, standard deviation.

[a]All subjects whose pharmacokinetic profile contained at least 4 consecutive data points with quantifiable concentrations.

repository corticotropin injection at all doses was lower than that at the lowest dose of synthetic $ACTH_{1-24}$ depot.

Dose-dependent increases in plasma concentrations of free cortisol were observed on study day 1 and study day 15 after administration of repository corticotropin injection (Figure 1) and on study day 1 and study day 5 after administration of synthetic $ACTH_{1-24}$ depot (Figure 2). Free cortisol levels returned to baseline within 24 hours after administration of repository corticotropin injection, whereas free cortisol levels remained elevated at 24 hours with synthetic $ACTH_{1-24}$ depot. Plasma-free cortisol concentrations after administration of oral methylprednisolone were consistent with the drug's known adrenal suppression (Figure S2).[19]

Simulated Baseline-Adjusted Free Cortisol Exposure at Clinically Relevant Doses of Synthetic $ACTH_{1-24}$ Depot

The population PK/PD model parameters and diagnostic plots are presented in Supplemental Tables S1 and S2 as well as Figures S3 and S4. Baseline-adjusted free cortisol exposure at the therapeutic dose of synthetic $ACTH_{1-24}$ depot (1 mg subcutaneously twice weekly) was derived through model-based simulation, as presented in Table 5.

Methylprednisolone and Prednisone Dose Equivalence

At steady state (day 15) following 16 mg once daily of methylprednisolone, the mean ± SD AUC_{τ} for free methylprednisolone was 61 651 ± 11 628 pg·h/mL). The free steroidogenic exposure at steady state ($AUEC_{\tau SG}$) produced by methylprednisolone at 16 mg once daily that was derived based on equation 2 was 305 ng·h/mL. The equivalent methylprednisolone doses for repository corticotropin injection and synthetic $ACTH_{1-24}$ depot were derived by applying equation 5 to the free cortisol exposure at steady state following dosing of either drug. At 40 IU twice weekly, 80 IU twice weekly, or 80 IU 3 times weekly of repository corticotropin injection, the weekly steroidogenic exposure was equivalent to what would be produced by 29, 46, or 88 mg of methylprednisolone. At 1 mg twice weekly of synthetic $ACTH_{1-24}$ depot, the weekly steroidogenic exposure was equivalent to 319 mg of methylprednisolone, as presented in Table 6. The daily and weekly prednisone-equivalent dose is also given in Table 6. Depending on the regimen, the steroidogenic exposure of repository corticotropin injection was approximately 3-fold to 11-fold lower than that of synthetic $ACTH_{1-24}$ depot. Clearly, repository corticotropin injection generates a much lower steroidogenic exposure than synthetic $ACTH_{1-24}$ depot at clinically relevant doses.

Table 4. Mean (SD) Cortisol Parameters After Administration of Repository Corticotropin Injection and Synthetic $ACTH_{1-24}$ Depot[a]

	Repository Corticotropin Injection						Synthetic $ACTH_{1-24}$ Depot							
	40 IU SC BIW		80 IU SC BIW		80 IU SC TIW		0.5 mg SC QD		0.75 mg SC QD		1 mg SC QD		1 mg IM QD	
Parameter	Day 1 (n = 12)	Day 15 (n = 12)	Day 1 (n = 12)	Day 15 (n = 11)	Day 1 (n = 12)	Day 15 (n = 12)	Day 1 (n = 14)	Day 5 (n = 14)	Day 1 (n = 14)	Day 5 (n = 14)	Day 1 (n = 14)	Day 5 (n = 14)	Day 1 (n = 13)	Day 5 (n = 13)
Total cortisol														
E_{max}[BA] (ng/mL)	141 (48)	155 (75)	178 (52)	202 (73)	168 (58)	217 (82)	346 (61)	540 (99)	352 (76)	574 (107)	335 (54)	575 (136)	353 (47)	617 (111)
$AUEC_{24}$[BA] (ng·h/mL)	1598 (1405)	2358 (1611)	2788 (1329)	3338 (1476)	2867 (1183)	3401 (1599)	5679 (1855)	7777 (2961)	6559 (1729)	10041 (2212)	5898 (1157)	9986 (2802)	6358 (996)	10611 (2420)
Free cortisol														
E_{max}[BA] (ng/mL)	24 (8)	27 (13)	30 (12)	39 (18)	28 (13)	46 (21)	52 (21)	135 (46)	57 (22)	136 (30)	58 (13)	159 (46)	58 (11)	165 (37)
$AUEC_{24}$[BA] (ng·h/mL)	157 (128)	280 (247)	316 (212)	442 (306)	314 (206)	561 (363)	860 (491)	1925 (1041)	1060 (424)	2407 (663)	992 (298)	2797 (978)	1030 (212)	2731 (753)

$ACTH_{1-24}$, the first 24 amino acids of adrenocorticotropic hormone; $AUEC_{24}$, area under the time effect curve from time 0 to 24 hours; BA, baseline-adjusted; BIW, twice weekly; E_{max}, maximum observed effect; IM, intramuscularly; QD, daily; SC, subcutaneously; SD, standard deviation; TIW, 3 times weekly.

[a]All subjects with at least 4 quantifiable cortisol data points following administration of 1 dose.

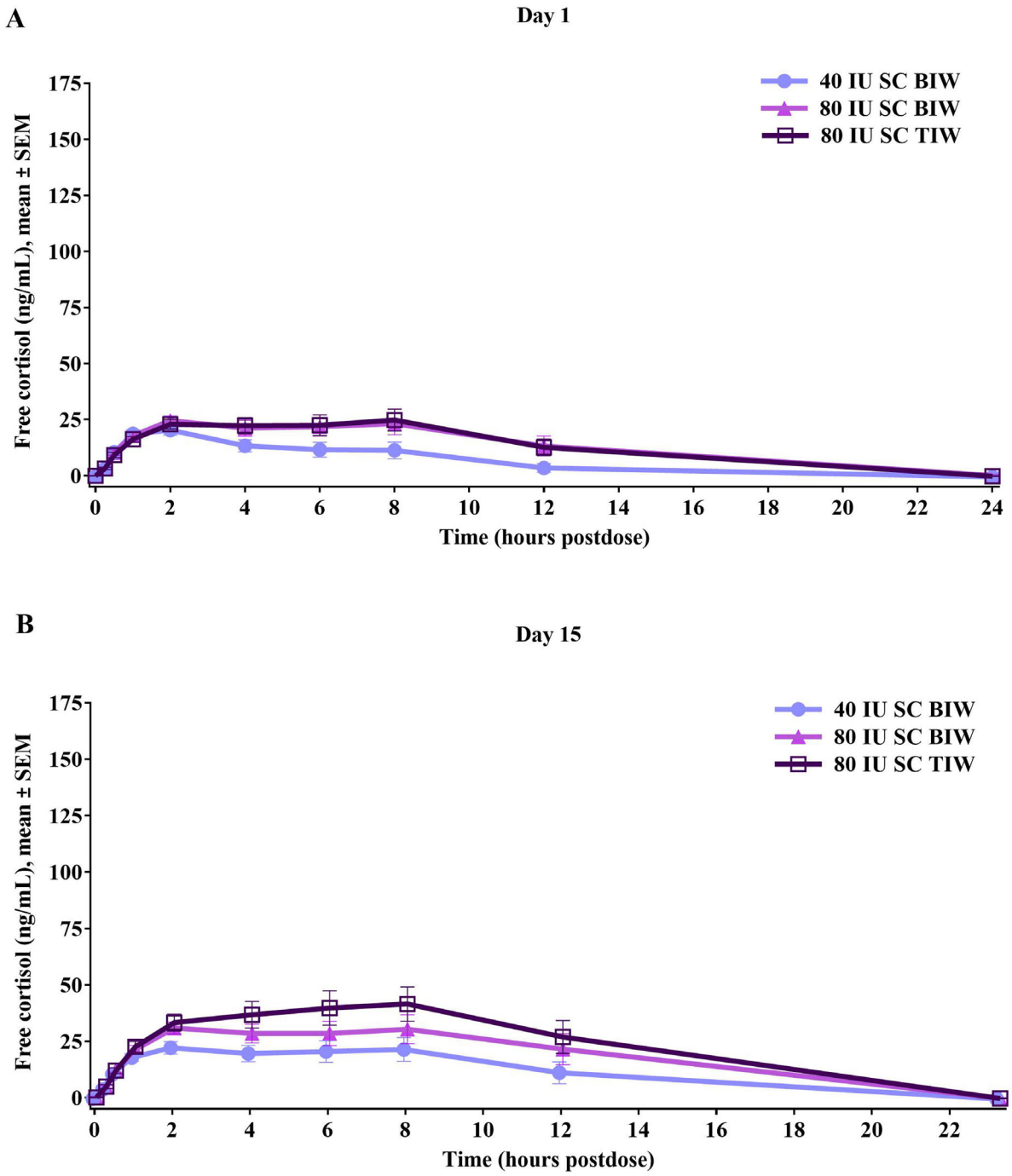

Figure 1. Change from baseline in plasma concentrations of free cortisol after administration of repository corticotropin injection on study day 1 (A) and study day 15 (B) for all subjects with at least 4 quantifiable cortisol data points following administration of 1 dose. BIW, twice weekly; SC, subcutaneously; SEM, standard error of the mean; TIW, 3 times weekly.

Discussion

Since the discovery of ACTH in the 1930s and ACTH sequence characterization in the 1950s, the variable steroidogenic effects of different formulations of ACTH and its analogues have been studied extensively.[14,20,21] The 2 phase 1 studies in healthy subjects described here further characterize the PK and PD of 2 ACTH analogues: repository corticotropin injection (a US FDA-approved complex mixture of ACTH analogues and other pituitary peptides) and synthetic $ACTH_{1\text{-}24}$ depot. Dose-dependent increases in cortisol production were observed with the administration of repository corticotropin injection and synthetic $ACTH_{1\text{-}24}$ depot. Cortisol levels returned to baseline

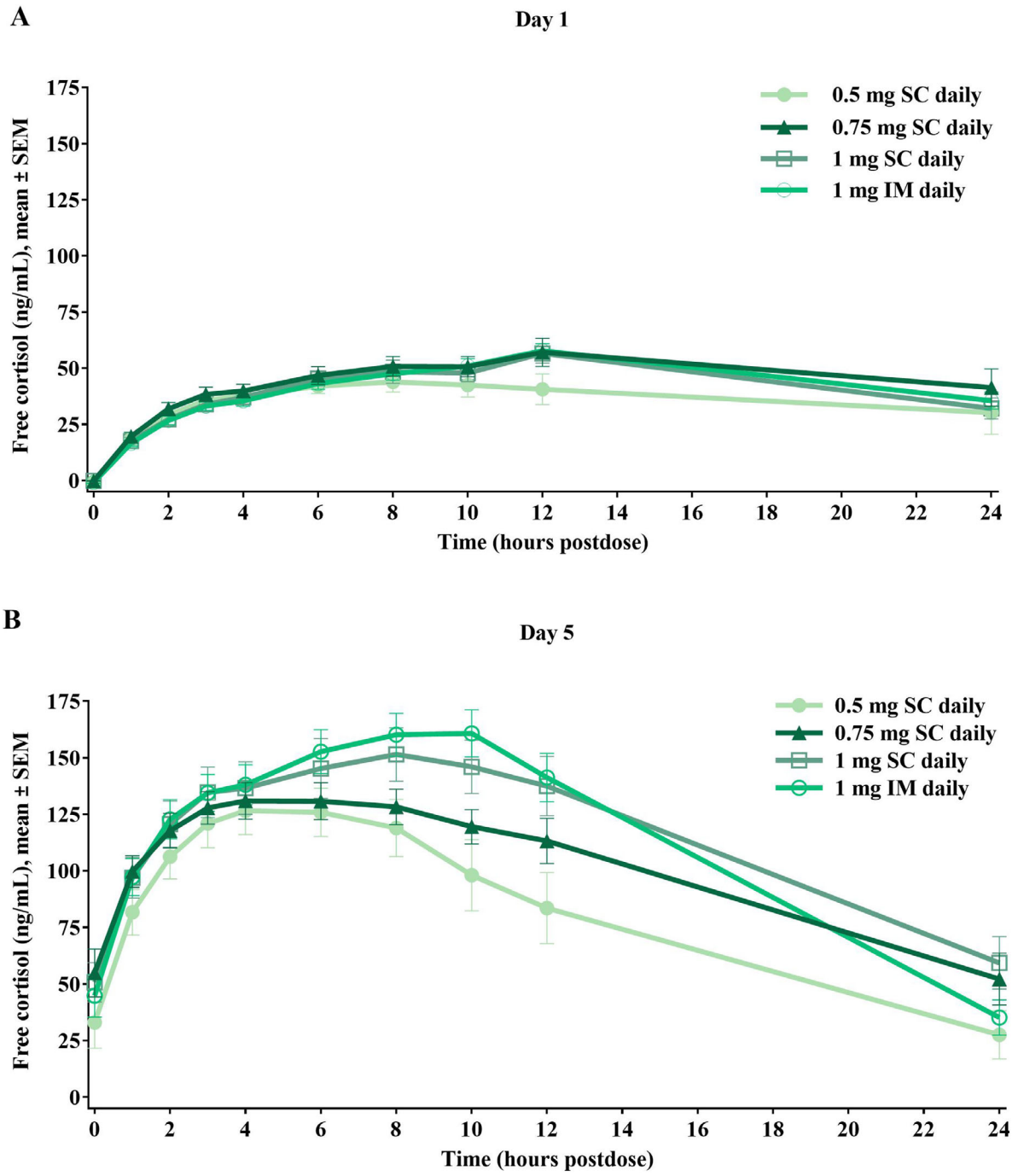

Figure 2. Change from baseline in plasma concentrations of free cortisol after administration of synthetic $ACTH_{1-24}$ depot on study day 1 (A) and study day 5 (B) for all subjects with at least 4 quantifiable cortisol data points following administration of 1 dose. $ACTH_{1-24}$, the first 24 amino acids of adrenocorticotropic hormone; IM, intramuscularly; SC, subcutaneously; SEM, standard error of the mean.

within 24 hours after administration of repository corticotropin injection. Although there was no accumulation, cortisol levels were higher on day 15 (steady state) than on day 1, suggesting increased MC2R sensitivity with repeated dosing.[22] Because cortisol levels remained elevated 24 hours after the administration of synthetic $ACTH_{1-24}$ depot, accumulation in cortisol exposure following daily dosing was observed.

A methylprednisolone cohort was included in the repository corticotropin injection study to derive the steroidogenic-equivalent dose. Using day 15 free cortisol exposure (steady state), a comparison of

Table 5. Simulated Mean (SD) Baseline-Adjusted Free Cortisol Exposure for Synthetic $ACTH_{1-24}$ Depot 1 mg SC BIW

	Week 1			Steady State		
	Day 1	Day 4	Week 1	Day 1	Day 4	Week 4
E_{max}[BA] (ng/mL)	56 (27)	57 (28)	57 (28)	57 (28)	57 (28)	57 (28)
AUEC[BA][a] (ng·h/mL)	1071 (530)	1163 (606)	5893 (3769)	1155 (598)	1170 (611)	6079 (3898)

$ACTH_{1-24}$, the first 24 amino acids of adrenocorticotropic hormone; AUEC, area under the time effect curve; $AUEC_{0-24}$, area under the time effect curve from time 0 to 24 hours; $AUEC_{0-168}$, area under the time effect curve from time 0 to 168 hours; BA, baseline-adjusted; BIW, twice weekly; E_{max}, maximum observed effect; SC, subcutaneously; SD, standard deviation.
[a]$AUEC_{0-24}$ for day 1 (dose 1) and day 4 (dose 2); $AUEC_{0-168}$ for week 1 and week 4.

Table 6. Mean Methylprednisolone and Prednisone Dose Equivalency of Repository Corticotropin Injection and Synthetic $ACTH_{1-24}$ Depot

Treatment	Weekly $AUEC_{ss}$ (ng·h/mL)	Equivalent to Weekly Methylprednisolone Dose (mg)[a]	Equivalent to Daily Methylprednisolone Dose (mg)	Equivalent to Weekly Prednisone Dose (mg)[b]	Equivalent to Daily Prednisone Dose (mg)
Repository corticotropin injection					
40 IU SC BIW (80 IU per week)	280[c] x 2 = 560	29	4	36	5
80 IU SC BIW (160 IU per week)	442[c] x 2 = 884	46	7	58	8
80 IU SC TIW (240 IU per week)	561[c] x 3 = 1683	88	13	110	16
Synthetic $ACTH_{1-24}$ depot 1 mg SC BIW (2 mg per week)	6079[d]	319	46	399	57

$ACTH_{1-24}$, the first 24 amino acids of adrenocorticotropic hormone; $AUEC_{24}$, area under the time effect curve from time 0 to 24 hours; $AUEC_{ss}$, free cortisol exposure; BA, baseline-adjusted; BIW, twice weekly; SC, subcutaneously; TIW, 3 times weekly.
[a]Calculated using equation 5.
[b]Calculated as 1.25 × methylprednisolone dose.
[c]Free cortisol levels returned to baseline prior to 24 hours; therefore, $AUEC_{ss}$ per week = day 15 $AUEC_{24}$[BA] × dosing times per week (BIW or TIW).
[d]Simulated weekly steady state $AUEC_{ss}$.

steroidogenic exposure across treatment groups found that at clinically relevant repository corticotropin injection treatment regimens, steroidogenic exposure corresponds to low to moderate daily corticosteroid doses.[15] Specifically, repository corticotropin injection doses of 40 IU subcutaneously twice weekly, 80 IU subcutaneously twice weekly, and 80 IU subcutaneously 3 times weekly correspond to about 5, 8, and 16 mg of total daily prednisone, respectively. In contrast, 1 mg subcutaneous synthetic $ACTH_{1-24}$ depot twice weekly was comparable to 57 mg of total daily prednisone, which is substantially higher than that of repository corticotropin injection at a therapeutically equivalent dose.[18]

Methylprednisolone is known to suppress the production of cortisol; thus, as expected, endogenous cortisol after the administration of methylprednisolone was negligible. In addition, methylprednisolone follows linear PK. Therefore, the steroidogenic concentrations for methylprednisolone were approximately proportional to the methylprednisolone plasma concentrations.[23] As such, its steroidogenic exposure is also dose-proportional.[24]

The population PK/PD model accurately described the $ACTH_{1-24}$ concentrations and free cortisol profiles of clinical doses of synthetic $ACTH_{1-24}$ depot. Model evaluation was conducted by diagnostic plots. During model development, only body weight was found to

be a significant covariate for clearance and volume of distribution for synthetic $ACTH_{1-24}$ depot. Therefore, the simulation accounted for the range of body weights in the data set (53-96 kg). As part of the evaluation of model simulation, the simulated free cortisol exposure of the 1-mg single dose of synthetic $ACTH_{1-24}$ depot was compared with the observed values. There was agreement between simulated and observed $ACTH_{1-24}$ and baseline-adjusted free cortisol levels (Supplemental Table S3).

Endogenous ACTH potently induces cortisol production[25]; however, the steroidogenic effects of different ACTH drug class formulations are known to be variable.[20,26] For example, a comparison of synthetic $ACTH_{1-24}$, synthetic $ACTH_{1-39}$, and a porcine-derived ACTH formulation (Organon, Inc.) demonstrated a higher corticosteroid response with the porcine-derived ACTH than with the other ACTH formulations.[26] These data are in contrast to our results showing that synthetic $ACTH_{1-24}$ depot was more steroidogenic than repository corticotropin injection. In addition, the corticosteroid response to the synthetic $ACTH_{1-24}$ depot used in our study and the $ACTH_{1-24}$ used by Brombacher et al were similar, suggesting that the porcine-derived ACTH formulation studied by Brombacher induces a greater corticosteroid response than repository corticotropin injection. However, a direct comparison between the porcine-derived ACTH formulation and repository corticotropin injection has not been reported. These findings highlight that differences in ACTH product preparation and purity yield formulations of ACTH that are not interchangeable.[27]

The lower steroidogenic exposure of repository corticotropin injection described here compared with synthetic $ACTH_{1-24}$ depot underscores nonclinical evidence suggesting that repository corticotropin injection exhibits anti-inflammatory effects independent of endogenous cortisol production.[9–11] Despite its low associated steroidogenic exposure, repository corticotropin injection has demonstrated efficacy in patients who previously have not responded to corticosteroids[1–6]; this further suggests that repository corticotropin injection exhibits a therapeutic effect independent of corticosteroid production. Given the side effect profile resulting from chronic use of exogenous corticosteroids,[28] the lower corticosteroid dose equivalence and reduced steroidogenic exposure of repository corticotropin injection suggest that it is a favorable alternative to corticosteroids for the treatment of inflammatory conditions.

These analyses have several limitations. This was an indirect comparison of 2 separate PK/PD studies with a model-based simulation to generate steroidogenic exposure for synthetic $ACTH_{1-24}$ depot at a clinically relevant dosing regimen. A direct study employing a clinically relevant dosing regimen of synthetic $ACTH_{1-24}$ depot to confirm the findings of this analysis would be valuable. In addition, future studies should evaluate the effect of these drugs on other mechanistic pathways distinct from MC2R activation and steroidogenic exposure. The clinical relevance of these data warrants further investigation in patients who have inflammatory conditions.

Conclusions

PK and PD studies in healthy subjects have demonstrated that repository corticotropin injection induces lower cortisol exposure compared with a synthetic $ACTH_{1-24}$ depot; synthetic $ACTH_{1-24}$ depot induces approximately 3-11 times the steroidogenic exposure of repository corticotropin injection at therapeutically equivalent doses. This indicates that repository corticotropin injection, as a complex mixture of pituitary peptides, has a pharmacologic effect that cannot be considered identical to other products in the ACTH drug class.

Conflicts of Interest

All authors were employees of Mallinckrodt Pharmaceuticals at the time of study completion. Technical and editorial support for this article was provided by MedLogix Communications, LLC, and funded by Mallinckrodt Pharmaceuticals.

Funding

This study was funded by Mallinckrodt Pharmaceuticals.

Data Sharing

The data sets generated and analyzed for this manuscript are not publicly available. Requests for additional information should be made to the corresponding author.

Author Contributions

All authors contributed to the conception or design of the work, acquisition, analysis, or interpretation of data, drafting the work or revising it critically for important intellectual content, and final approval of the version to be published.

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Supplemental Information

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