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Altered Methylprednisolone Pharmacodynamics in Healthy Subjects With *Histamine N-Methyltransferase* C314T Genetic Polymorphism

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Abstract

This study investigated the potential differences in methylprednisolone pharmacodynamics between healthy subjects with different *histamine N-methyltransferase* (*HNMT*) C314T genotypes. Six individuals with C/C genotype and 4 with C/T genotype were administered a single intravenous dose of methylprednisolone 0.6 mg/kg ideal body weight in a randomized 2-period manner. Methylprednisolone plasma concentrations were fitted with a 1-compartment model. Cortisol and whole blood histamine suppression were assessed by indirect response models, with circadian baseline cortisol analyzed by Fourier analysis. The area between the baseline and effect curve and the area under the effect versus time curve suppression ratiowere used to characterize plasma histamine suppression. Methylprednisolone pharmacokinetics and plasma and whole blood histamine suppression were similar between the 2 genotype groups. Median nadir of cortisol and the 50% inhibitory concentration for cortisol were significantly higher in subjects with C/T genotype than those with C/C genotype (P = .031 and .033, respectively, Wilcoxon rank sum test). Subjects who are heterozygous for the T314 variant allele thus appeared less sensitive to the suppressive effects of methylprednisolone on cortisol secretion.

Keywords

Corticosteroids; methylprednisolone pharmacodynamics; cortisol suppression; histamine suppression; *HNMT* polymorphism

Histamine is an important mediator in the body that is involved in the regulation of numerous physiologic and pathophysiologic processes including gastric acid secretion, central nervous system functioning, bronchial asthma, and hypersensitivity reactions. It is formed by decarboxylation of histidine, a metabolic pathway that is catalyzed by histidine

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decarboxylase (HDC)[1] and is metabolized by diamine oxidase and histamine N-methyltransferase (HNMT).[2] Histamine N-methyltransferase catalyzes the N-methylation of histamine, which was suggested to be the major significant pathway for the biotransformation of histamine in bronchial epithelium and the brain.[3–5] Interindividual variation of HNMT activity has been demonstrated in whites and Han Chinese.[6,7] This variability can be explained in part by a C to T transition at nucleotide 314 (exon 4) of the *HNMT* gene, causing an amino acid change of threonine to isoleucine at codon 115, which results in low immunoreactive HNMT protein and activity.[8] Previously, HNMT activity in red blood cell lysate was found to be lower in persons who are heterozygous for the T314 allele than in those with homozygous wild-type genotype.[7,8]

Corticosteroids are widely used for their anti-inflammatory and immunosuppressive effects in various diseases including allergic diseases such as asthma, urticaria, rhinitis, and anaphylaxis. These agents elicit a number of pharmacodynamic responses including suppression of cortisol secretion, cell trafficking, and whole blood histamine (WBH) concentrations. The suppressive effects of corticosteroids on WBH are well characterized by an indirect response model.[9–13] On the contrary, little is known about the pharmacodynamic responses of plasma histamine after corticosteroid administration. It has been reported that for asthmatic patients with high baseline plasma histamine, levels of plasma histamine were reduced by approximately 50% at day 3 and day 14 after the initiation of corticosteroid therapy.[14]

Because histamine concentrations are suppressed by corticosteroids and HNMT activity is reduced as a result of C314T polymorphism, we suspected that an alteration in histamine metabolism or concentration as a result of *HNMT* C314T might cause a change in plasma or WBH response to steroid administration. Therefore, we sought to assess possible differences in methylprednisolone pharmacodynamics in subjects with different *HNMT* C314T genotypes. Because cortisol suppression is a well-established pharmacodynamic model, we also opted to determine methylprednisolone pharmacokinetics and plasma cortisol dynamics in the study as well.

METHODS

This study was approved by the Institutional Review Board of Mercer University and was performed at the Center for Clinical Research, Mercer University Southern School of Pharmacy, Atlanta, Georgia. Written informed consent was obtained from all subjects before any study procedures were performed.

Subjects

Forty-eight healthy volunteers (36 males, 12 females), with mean ± SD age of 26 ± 5 years, were screened for the presence of *HNMT* C314T polymorphism by a polymerase chain reaction–restriction fragment length polymorphism assay as previously described.[15] Genotyping revealed that 11 subjects carry the variant T314 allele. Ten were heterozgyous C/T (8 whites, 1 Chinese, 1 descendent of Middle Eastern origin), and 1 was homozygous T/T (Pakistani). Two of these subjects were taking prescription drugs and were not eligible for the pharmacokinetic/pharmacodynamic study. Five, including the one with homozygous

T/T genotype, were unable or refused to participate. Only 4 heterozygotes (3 men, 1 woman; all whites) participated and completed the pharmacokinetics study. Six other subjects with the homozygousC/C genotype (allmen; 5 white, 1 Chinese) were randomly chosen and completed the study. These 10 subjects were 21 to 33 years of age (mean, 24 ± 3.8 years) and were within 125% of ideal body weight (mean weight, 74.2 ± 7.35 kg).

The health status of the subjects was assessed by medical and drug history, physical examination, electrocardiogram, urinalysis, complete blood chemistry (liver function tests and renal function tests), and hematologic (complete blood count) profile before the study. None of the subjects were smokers, had excessive alcohol intake, or had a history of underlying major diseases. One subject had a history of seasonal allergy. However, he did not have an active disease and was not taking any antihistamines or steroids at the time of the study. No subjects had documented allergy to corticosteroids or participated in a similar study within the past month. Subjects were not taking chronic medications and had not used steroid hormones, nonsteroidal anti-inflammatory drugs, antihistamines, and medications that are known to have antihistaminic effects or alter methylprednisolone metabolism. Drugs such as acetaminophen were permitted on the approval of the study investigator or physician. One woman participated in this study. She was not pregnant and had regular monthly menstruation. She was on oral contraceptives but stopped taking the pills 10 days before the first 24-hour baseline study. Fourteen days later, the drug study was performed. No significant adverse reactions occurred in the study.

Procedures

The pharmacokinetics study consisted of a baseline phase (24 hours, no drug) and a methylprednisolone phase (32 hours, with drug) separated by a 2-week washout period. During the baseline phase, plasma cortisol and histamine and WBH concentrations were obtained in the absence of methylprednisolone. Nine milliliters of blood were drawn from each subject every 2 hours for 24 hours. Four milliliters of the blood were aliquoted into a plastic EDTA-containing tube, which was immediately placed on ice after collection. The tube was then centrifuged at 3000 revolutions perminute at 4°C for 15 minutes. Plasma was aliquoted into cryovials, frozen, and stored at −80°C for later determination of plasma histamine concentration. The remaining 5mL of blood were collected into a plastic heparincontaining tube at room temperature. One milliliter of the heparinized whole blood was transferred into cryovials, frozen, and then stored at −80°C for analysis of WBH. The rest of the blood was centrifuged at 3000 revolutions per minute at 4°C for 15 minutes. Plasma was aliquoted and stored at −80°C for later determination of cortisol concentration.

During the methylprednisolone phase, each subject was administered an intravenous bolus dose of 0.6mg/ kg (ideal body weight) methyl- prednisolone sodium succinate (Solu-Medrol, Upjohn, Kalamazoo, Mich) over 2 minutes. Ten milliliters of blood were drawn at 0, 0.25, 0.5, 1, 1.5, 2, 3, and 4 hours and then every 2 hours until 24 hours, 28, and 32 hours. Blood was processed as described above.

Male subjects were randomized to initiate either the baseline or methylprednisolone phase of the study. The female subject underwent the baseline phase during the follicular phase of her menstrual cycle, followed by the methylprednisolone phase during the luteal phase 2weeks

later. All subjects fasted from 8:00 PM the evening before and for 2 hours after receiving methylprednisolone. Each study phase began at 6:00 AM, at which time a 20-gauge angiocatheter was placed in a suitable arm vein to facilitate blood collection. The catheter was kept patent with diluted heparin (10 U/ mL) solution frequently.Vital signs weremeasured and monitored every hour for the first 5 hours of each study phase.

Analytical Assays

Plasma cortisol and methylprednisolone concentrations were determined simultaneously by a normal phase high-performance liquid chromatography method as reported previously.[16] The lower limit of quantitation (LLQ) was 5 ng/mL for both cortisol and methylprednisolone. The intraday and interday coefficients of variation for low, median, and high quality control samples were all below 7% for cortisol and below 4% for methylprednisolone. For the pharmacodynamic analysis of cortisol, concentrations that fell below the LLQ were set to half the limit of quantitation (2.5 ng/mL). Methylprednisolone concentrations falling below the LLQ were excluded from data analysis.

Plasma and WBH concentrations were determined by a commercial enzyme immunoassay kit (Immunotech, Marseille, France), according to the manufacturer's instructions. The standard curve was fitted with a 4- parameter logistic function using SigmaPlot 8.0 (SPSS Inc, Chicago, Ill). The LLQ was 0.5 nM (0.056 ng/mL). Concentration units were converted from nMto ng/mL by multiplying by 0.111. The intraday and interday coefficients of variation for the quality control sample were 9.24% and 10.6%, respectively. All samples were assayed in duplicate.

Pharmacokinetics

Methylprednisolone concentration (C_{MP}) versus time (t) curves after intravenous administration were fitted by a 1-compartment model with 1/y weighting using WinNonlin 4.1 (Pharsight Corp, Mountain View, Calif) according to

$$C_{MP}=\frac{D}{V_d}\bullet e^{-k_{el}\bullet t} \quad (1)$$

where D is the dose administered, V_d is the volume of distribution, and k_{el} is the first-order elimination rate constant. Half-life ($t_{½}$) was calculated as $0.693/k_{el}$, clearance (CL) as a product of k_{el} and V_d, and the area under the concentration versus time curve (AUC) as D/CL.

Pharmacodynamics

Cortisol suppression—Endogenous cortisol concentrations (Cort) in the blood follow a circadian-episodic profile under normal physiologic conditions. The rate of change of cortisol concentration (dCort/dt) can be described by a periodic time-dependent production function $k_{in}(t)$ and the first-order elimination rate constant k_{out}:

$$\frac{dCort}{dt}=k_{in}(t)-k_{out}\bullet Cort. \quad (2)$$

After the administration of methylprednisolone, the inhibitory effect on cortisol secretion can be described by an indirect response model (Figure 1)[17]:

$$\frac{dCort}{dt}=k_{in}(t)\bullet(1-\frac{I_{\max}\bullet C_{MP}}{C_{MP}+IC_{50}})-k_{out}\bullet Cort \quad (3)$$

where I_{max} is the maximum inhibitory factor and IC_{50} is the concentration of methylprednisolone producing 50% suppression of cortisol circadian secretion. I_{max} was assumed to be equal to 1 and C_{MP} was generated using equation 1.

The circadian baseline cortisol concentrations can be approximated by the sum of a Fourier series of N harmonics[18]:

$$Cort(t)=a_0+\sum_{1}^{N}[a_n\bullet\cos(\frac{2\pi nt}{T})+b_n\bullet\sin(\frac{2\pi nt}{T})] \quad (4)$$

where T is the period and the constants a_0, a_n, and b_n, with n = 1,2, … are the Fourier coefficients of the function Cort(t). The bracketed term is referred to as the nth harmonic. An approximation of $k_{in}(t)$ can then be resolved as[18]

$$k_{in}(t)=A_0+\sum_{1}^{N}[A_n\bullet\cos(\frac{2\pi nt}{T})+B_n\bullet\sin(\frac{2\pi nt}{T})] \quad (5)$$

where $A_0 = a_0 \bullet k_{out}$, $A_n = a_n \bullet k_{out} + b_n \bullet 2\pi nt/T$, and $B_n = b_n \bullet k_{out} - a_n \bullet 2\pi nt/T$.

Fourier analysis was used to analyze baseline cortisol concentrations (placebo data from the 24-hour study) using the Fortran program FOURPHARM.[18] All data were analyzed based on a 24-hour period and the Fourier coefficients (a_0, a_1, b_1, …, a_N, and b_N) were determined by square L^2 norm approximation according to equation 4.[18] The number of harmonics was chosen based on the percentage of contribution of each harmonic to the baseline function.[18] The cortisol input function $k_{in}(t)$ was recovered and expressed in terms of Fourier coefficients and k_{out} (equation 5). This $k_{in}(t)$, together with C_{MP} calculated by equation 1, was then used to model cortisol concentrations after methylprednisolone administration (drug treatment data from the 32-hour study) according to equation 3. The pharmacodynamic parameters k_{out} and IC_{50} were estimated using the ADAPT II program (Biomedical Simulations Resource, University of Southern California, Los Angeles, Calif) and the maximum likelihood estimation procedure.

Twenty-four-hour area under the effect versus time curves $(AUEC_0^{24})$ for cortisol concentrations during the baseline $(AUEC_{0\ BL}^{24})$ and methylprednisolone phase $(AUEC_{0\ MP}^{24})$ were calculated with the linear trapezoidal method using SigmaPlot 8.0 (SPSS Inc, Chicago, Ill). These areas were used to calculate the AUEC suppression ratio ($AUEC_{SR}$) and the area between the effect curves (ABEC) according to

$$AUEC_{SR}=\frac{AUEC_{0\ MP}^{24}}{AUCE_{0\ BL}^{24}} \quad (6)$$

$$ABEC=AUEC_{0\ BL}^{24}-AUEC_{0\ MP}^{24}. \quad (7)$$

$AUEC_{SR}$ and ABEC were used to assess the 24-hour suppressive effects of methylprednisolone. Greater suppression is indicated by a smaller $AUEC_{SR}$ as well as a larger ABEC. Paired data for the 24-hour time point were not available for 3 subjects. The concentrations stimulated from the model were used to calculate $(AUEC_0^{24})$ for these missing points.

Whole blood histamine suppression—The WBH suppression model was used previously to describe the suppression of basophil trafficking after corticosteroid administration.[11,12] The rate of change ofWBH concentration at baseline was

$$\frac{dWBH}{dt}=k_r^0-k_H\bullet WBH \quad (8)$$

in which k_H is the rate constant for overall disappearance of histamine from blood, and k_r^0 is the zero-order rate constant for histamine production. The inhibitory effect of methylprednisolone is described by

$$\frac{dWBH}{dt}=k_r^0\bullet\left(1-\frac{I_{\max}\bullet C_{MP}}{C_{MP}+IC_{50}}\right)-k_H\bullet WBH \quad (9)$$

where IC_{50} represents the concentration of methylprednisolone causing 50% inhibition of k_r^0 (Figure 2). Again, I_{max} was assumed to be equal to 1. The WBH concentrations at baseline and after methylprednisolone were simultaneously fitted to equation 8 and 9 using ADAPT II and the maximum likelihood estimation procedure. The pharmacodynamic parameters k_r^0, k_H, and IC_{50} were estimated and $(AUEC_0^{24})$, $AUEC_{SR}$, and ABEC were calculated.

Plasma histamine suppression—Changes in plasma histamine concentrations at baseline and after methylprednisolone were highly variable and did not exhibit a consistent pattern. Therefore, pharmacodynamic modeling was not performed, and the only parameters calculated were $AUEC_{SR}$ and ABEC. For the 3 subjects who had missing data, the AUEC between time 0 and the time for the last measurable concentration (either 20 or 22 hours) was calculated for both phases.

Statistical Analyses

The pharmacokinetic and pharmacodynamic parameters including $AUEC_{SR}$ and ABEC values were com-pared between subjects with C/C genotype and those with C/T genotype by the Wilcoxon rank sum test. Statistical significance was set at $P \leq .05$, and all analyses were performed using the statistical software Statistica 6.1 (StatSoft, Tulsa, Okla).

RESULTS

Pharmacokinetics

The mean plasma methylprednisolone concentration versus time curves for subjects with different genotypes are depicted in Figure 3. All curves displayed monoexponential disposition and were well described by a 1-compartment model. Table I summarizes the pharmacokinetic parameter values for methylprednisolone for subjects with both genotypes. Whereas a slightly smaller median Vd was observed for subjects who are heterozygous for the T314 variant allele, no significant differences in pharmacokinetic parameter values were found between the 2 groups.

Pharmacodynamics

Cortisol suppression—Figure 1 depicts the plasma cortisol concentration versus time profiles during baseline and methylprednisolone phases for a subject with C/C genotype (top panel) and a selected subject with C/T genotype (bottom panel). The shape of the cortisol profiles from all other subjects resembled that of the subjects shown. In general, cortisol displayed normal circadian rhythm at baseline, with peak concentration at late morning and trough concentration at midnight. After methylprednisolone dosing, cortisol concentrations rapidly declined to reach a nadir at about 10 hours. Cortisol remained suppressed for a prolonged period of time until 20 to 22 hours, followed by a rapid return toward baseline at about 24 to 28 hours. The cortisol profile after drug administration was different for 1 subject with the C/T genotype. For this subject, cortisol concentrations after methylprednisolone rapidly dropped and reached a nadir at 6 hours and then quickly rebounded to a level close to the physiologic baseline at 10 hours. Thereafter, cortisol concentrations declined slowly from 10 to 22 hours, followed by a quick return toward baseline at 32 hours.

Figure 4 illustrates the changes of mean plasma cortisol concentration after methylprednisolone for the 2 groups of subjects. It was observed that the overall pattern of changes was similar between the 2 groups, but the suppressive effects were stronger in subjects who have C/C genotype. The differences in nadir cortisol concentration and the IC_{50} for cortisol suppression between the 2 genotype groups are depicted in Figure 5. At nadir, cortisol concentrations for all subjects with C/C genotype were less than 15 ng/mL. The absolute nadir of cortisol suppression was higher in subjects who are heterozygous for the T314 allele, with 3 of 4 subjects having nadir cortisol concentration greater than 19 ng/mL. The median (range) cortisol nadir reached for subjects with C/C genotype was 2.81 ng/mL (range, 2.50–11.6 ng/mL), whereas the median was 22.5 ng/mL (range, 6.22–27.4 ng/mL) (P = .031) for those with C/T genotype.

Table II describes the pharmacodynamic parameter estimates for cortisol suppression. The parameters k_{out}, $AUEC_{SR}$, and ABEC were similar, whereas IC_{50} was different between the 2 groups [1.87 ng/mL (range, 0.980–9.76 ng/mL) vs 34.0 ng/mL (range, 2.45–147); P = .033]. As seen in Figure 5, IC_{50} estimates were quite consistent among subjects with C/C genotype, and they were all low, whereas wide variability was found among subjects with

the C/T genotype. With the exception of 1 subject, the IC_{50} for all subjects with the C/T genotype was higher than that for subjects with C/C genotype.

Whole blood histamine suppression—The WBH concentration versus time profiles for selected study participants with C/C and C/T genotypes are shown in Figure 2. Generally, baseline values were relatively constant over 24 hours. After methylprednisolone, WBH concentrations decreased to a minimum at 8 to 12 hours and then slowly increased and returned to the baseline level at 24 to 32 hours. Table III summarizes the pharmacodynamic parameter values for WBH suppression for all subjects. No differences were found for the 2 groups.

Plasma histamine suppression—The concentrations of plasma histamine at baseline appeared to fluctuate between 0.11 and 1.1 ng/mL, but no consistent patterns could be observed. This random fluctuation remained after methylprednisolone. However, an overall suppression was seen, with individual plasma histamine concentrations varying irregularly between 0.08 and 0.8 ng/mL. The pharmacodynamic parameters $AUEC_{SR}$ and ABEC were used to characterize these profiles, and their values for subjects with different *HNMT* genotypes are listed in Table III. Although no difference was observed in ABEC, $AUEC_{SR}$ tended to be lower in subjects with the C/C genotype ($P = .055$).

DISCUSSION

This study is the first to investigate the pharmacokinetics/pharmacodynamics of methylprednisolone in healthy subjects with or without *HNMT* C314T polymorphism. Because no prior information was available for the differences in methylprednisolone pharmacokinetics/pharmacodynamics between subjects with different *HNMT* genotypes, the study was intended to be a pilot evaluation assessing the differences between the 2 genotype groups. No differences were observed for CL, Vd, $t_{½}$, and AUC between subjects with C/C and C/T genotypes. This finding is not unexpected, as no underlying mechanisms are currently known to be responsible for potential alterations in methylprednisolone disposition by the *HNMT* gene.

Using WBH concentrations as surrogate markers, the cell trafficking model describes the circulation of basophils between the central blood and extravascular compartments.[9–12] This model is based on observations that suppression of WBH concentrations after corticosteroid administration was parallel to that of numerical basophil counts[19,20] and the assumption that the number of blood basophils is directly proportional to WBH concentration. However, previous studies have shown that declines in WBH concentrations were slightly greater than those of basophil counts,[19,20] indicating that the suppression of WBH by corticosteroids was not entirely attributed to the efflux of basophils from blood and that additional mechanisms such as a steroid-mediated alteration in histamine metabolism might also be involved.

Our results showed that pharmacodynamic parameter values for WBH suppression were not different between subjects with the 2 genotypes, suggesting that *HNMT* C314T polymorphism was not significantly correlated with the changes of WBH. This finding is consistent with previous findings showing association of HDC but not HNMT with

histamine metabolism in mast cells and basophils.[21–25] Furthermore, a recent study demonstrated that HDC messenger RNA(mRNA) and activity were downregulated by dexamethasone in adrenalectomized rat lung.[26] Levels of HDC mRNA declined over 0 to 6 hours after a single bolus dose of dexamethasone and then returned near to baseline at around 24 hours. These temporal alterations in transcripts resemble the time course of WBH changes in humans, suggesting that reductions in histamine biosynthesis as a result of HDC downregulation can occur.

This report describes for the first time the pharmacodynamic responses of plasma histamine to methylprednisolone. Unlike WBH, no consistent suppression profile could be identified. This difference in behavior suggests that different processes might be involved in the regulation of histamine in plasma versus whole blood. Previous studies have demonstrated inhibition of histamine release from basophils by glucocorticoids[27,28]; the suppression of plasma histamine in our subjects may possibly be related to this inhibition.

In this study, we used Fourier analysis to characterize the circadian rhythm of cortisol concentration at baseline. One harmonic was chosen and was used for all subjects. After methylprednisolone, cortisol concentrations were fitted reasonably well for all subjects except for one heterozygote whose IC_{50} (147 ng/mL) was the highest among all (Figure 1, bottom panel). In this subject, cortisol concentrations rapidly declined 71% from 95.7 ng/mL to a nadir of 27.3 ng/mL within 6 hours, followed by a quick rebound shortly after the nadir was reached. This degree of suppression was only moderate when compared to the 93% to 97% drop in cortisol for subjects with the wild-type genotype. The methylprednisolone concentration at 6 hours was 124 ng/mL, which was below the IC_{50}. Therefore, it could no longer maintain cortisol suppression, and the normal circadian rhythm of cortisol resumed. The fitted curve for this subject could not capture all the changes in cortisol concentration, and the fitting can possibly be better individualized. However, we chose to use one fixed model to allow modeling of all subjects in a consistent manner.

When we first designed the study, we anticipated that there might be differences in histamine pharmacodynamics between subjects with different *HNMT* genotypes. Surprisingly, differences in cortisol response to methylprednisolone were observed. At first glance, cortisol suppression appeared to be similar among all subjects. Careful examination of the data and statistical analysis revealed that the median nadir of cortisol suppression was in fact higher in subjects with the C/T genotype, indicating that the suppressive effects of methylprednisolone on cortisol secretion were less pronounced in these subjects. The slight differences in cortisol nadir between the 2 groups, however, did not contribute to a difference in median $AUEC_{SR}$ or ABEC. It is likely that the subtle differences in cortisol were obscured during the calculation of AUEC values, thus making $AUEC_{SR}$ and ABEC insensitive measurements. On the other hand, mathematical modeling of cortisol concentrations provided a sensitive technique to detect the differences in methylprednisolone pharmacodynamics. The differences in cortisol suppression were confirmed by the higher median IC_{50} estimated for subjects with the C/T genotype. Of note, IC_{50} was highly variable in this genotype group, implying that cortisol responses to methylprednisolone could potentially be associated with additional molecular factors. Recently, several polymorphisms (T-1637C, T-463C, A939G, and A1097T) in the

noncoding region of the *HNMT* gene have been reported to be associated with altered HNMT activity.[7] It is possible that these polymorphisms might also be involved in the regulation of *HNMT* and thus correlated with the suppression of cortisol by corticosteroids. Because of the small sample size, high variability of IC_{50}, low penetrance of the trait, and examination of heterozygotes only in this study, further investigation is necessary to confirm our current findings.

Previous studies have shown that cortisol and adrenocorticotropic hormone (ACTH) secretions were stimulated by intravenous and intracerebroventricular injection of histamine in animals[29–33] and suggested that these stimulatory effects were mediated via the activation of the H1 and H2 receptors[33–36] through the induction of corticotrophin releasing hormone (CRH)[37–39] and proopiomelanocortin (POMC)-derived peptide expressions.[40] The responses of ACTH to centrally administered histamine, however, were prevented by pretreatment with glucocorticoids,[40] which are known to inhibit POMC mRNA synthesis and expression in the anterior pituitary.[41–43] Because methylation was suggested to be the main pathway for histamine inactivation in the brain[4] and HNMT activity was found to be highest in the hypothalamus,[44,45] we speculate that the smaller suppression of cortisol in subjects with the C/T genotype might be related to a higher histamine concentration in the histaminergic neurons of the hypothalamus, which resulted in higher expressions of CRH and POMC through the activation of H1 and H2 receptors. This attenuated the inhibitory effects of methylprednisolone on POMC expression and ACTH secretion, subsequently leading to lower suppressive effects on cortisol secretion. It remains to be determined the role of HNMT in histamine catabolism in the brain and the mechanisms of steroid-induced cortisol suppression in subjects with *HNMT* C314T polymorphism.

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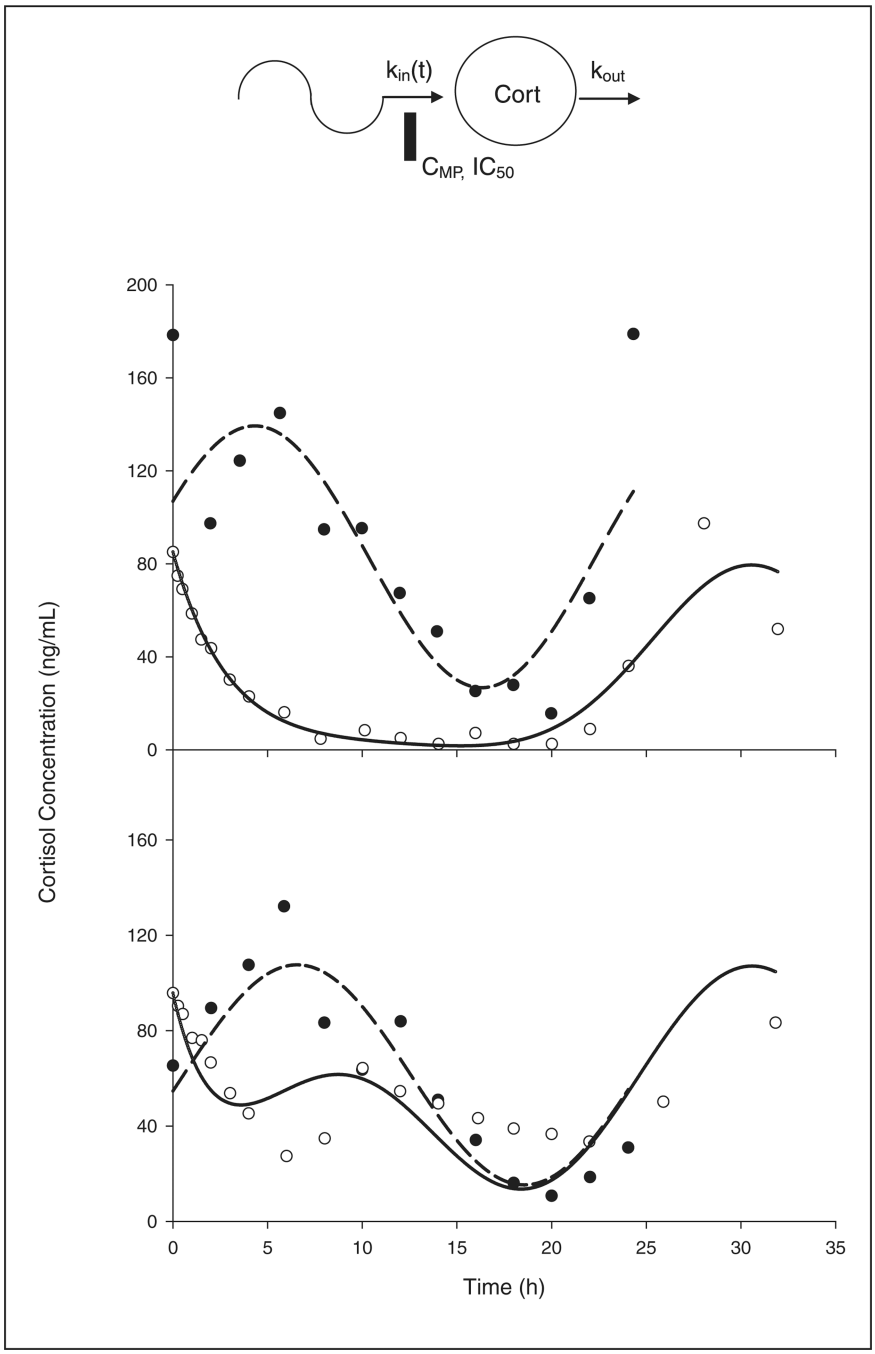

Figure 1.

Plasma cortisol concentration versus time profiles for subjects with C/C (top panel) and C/T genotypes (bottom panel). Symbols represent observed data and lines represent fitted curves obtained by Fourier analysis and the pharmacodynamic model shown at the top. The baseline phase is displayed by closed circles and dotted lines. The methylprednisolone phase is depicted by open circles and solid lines.

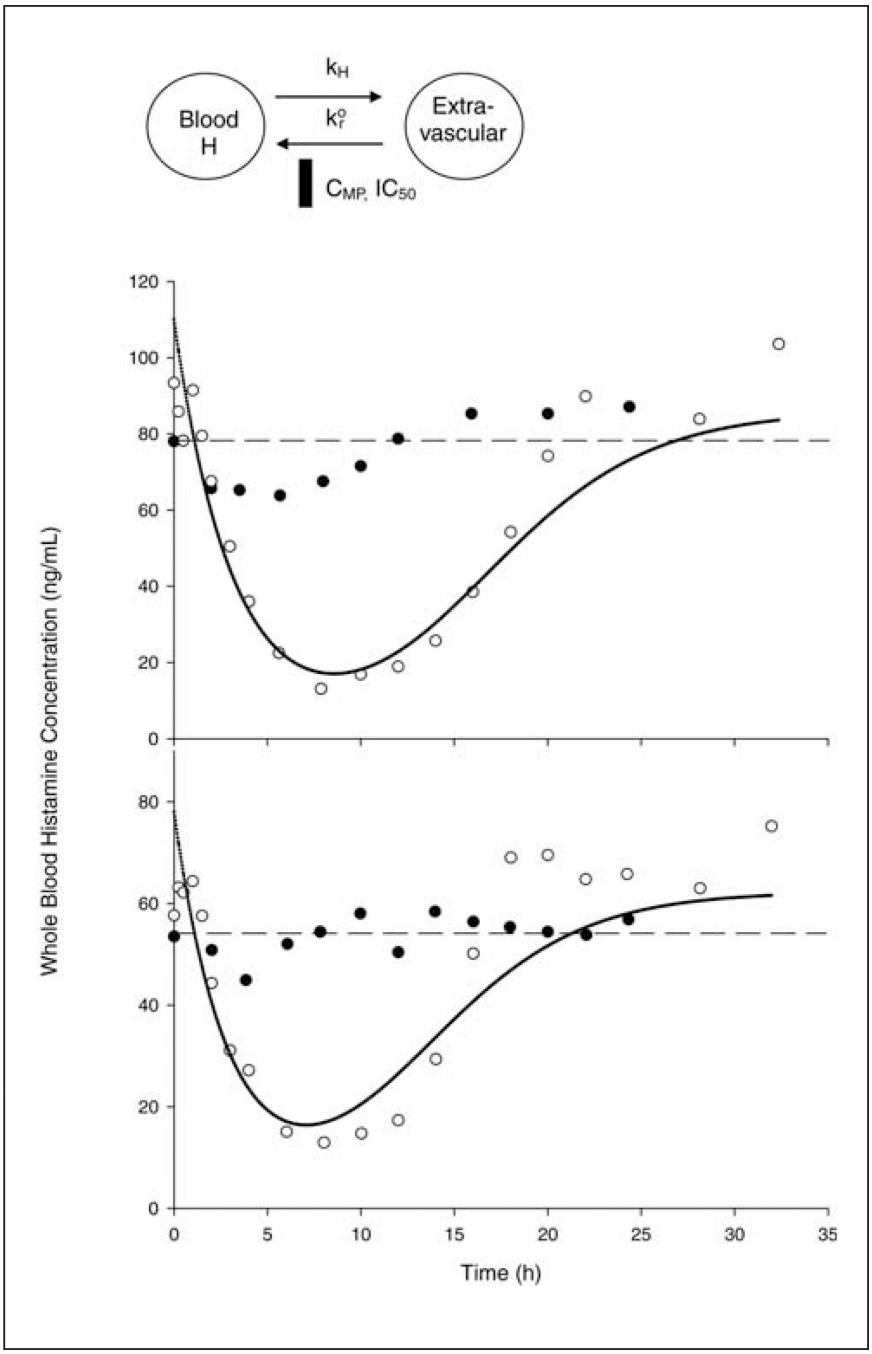

Figure 2.

Whole blood histamine concentration versus time profiles for subjects with C/C (top panel) and C/T genotypes (bottom panel). Symbols represent observed data and lines represent fitted curves. The baseline phase is displayed by closed circles and dotted lines. The methylprednisolone phase is depicted by open circles and solid lines.

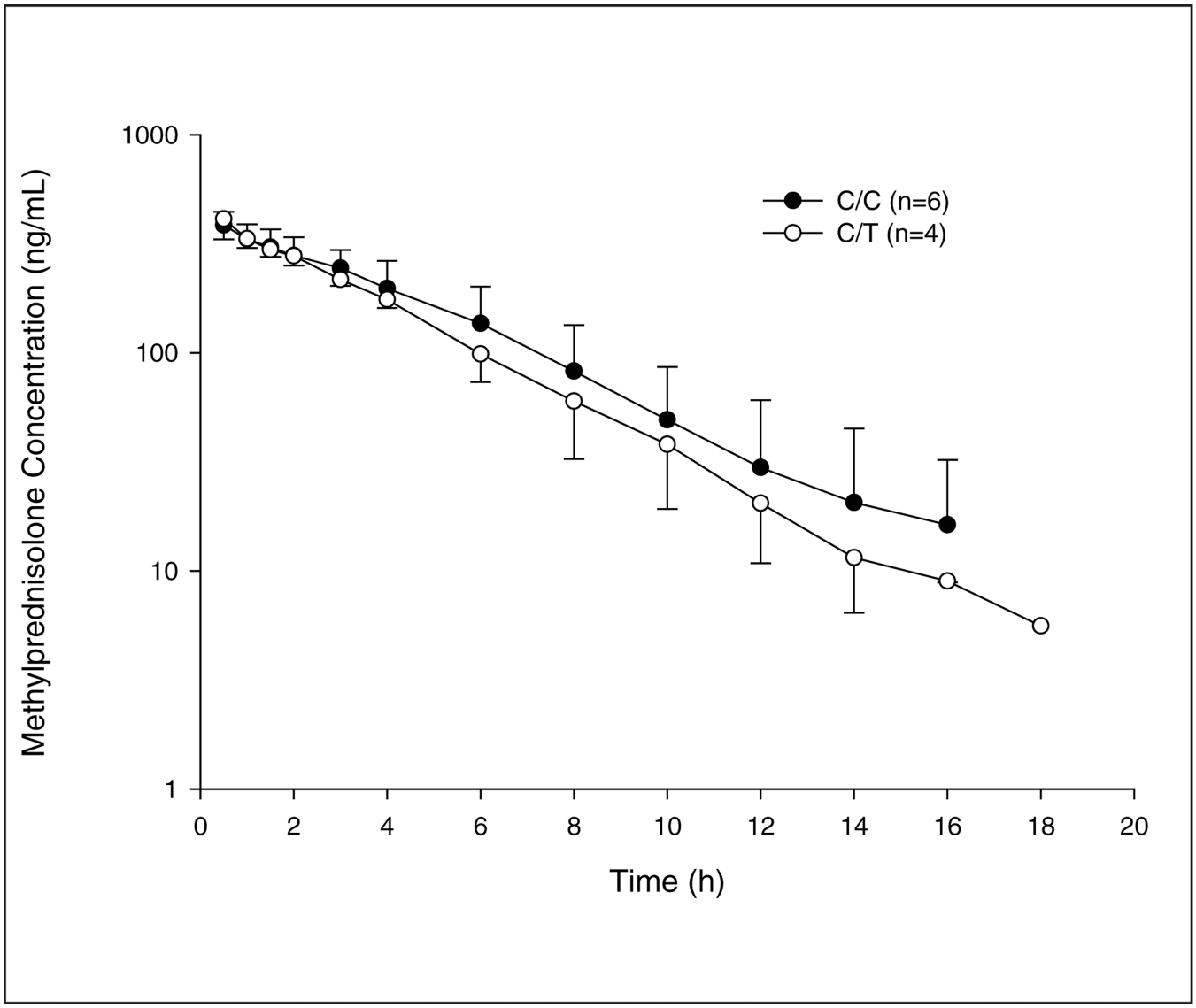

Figure 3.

Mean (±SD) plasma methylprednisolone concentration versus time curves for subjects who are homozygous (C/C, closed circles) and heterozygous (C/T, open circles) for the wild-type C314 allele.

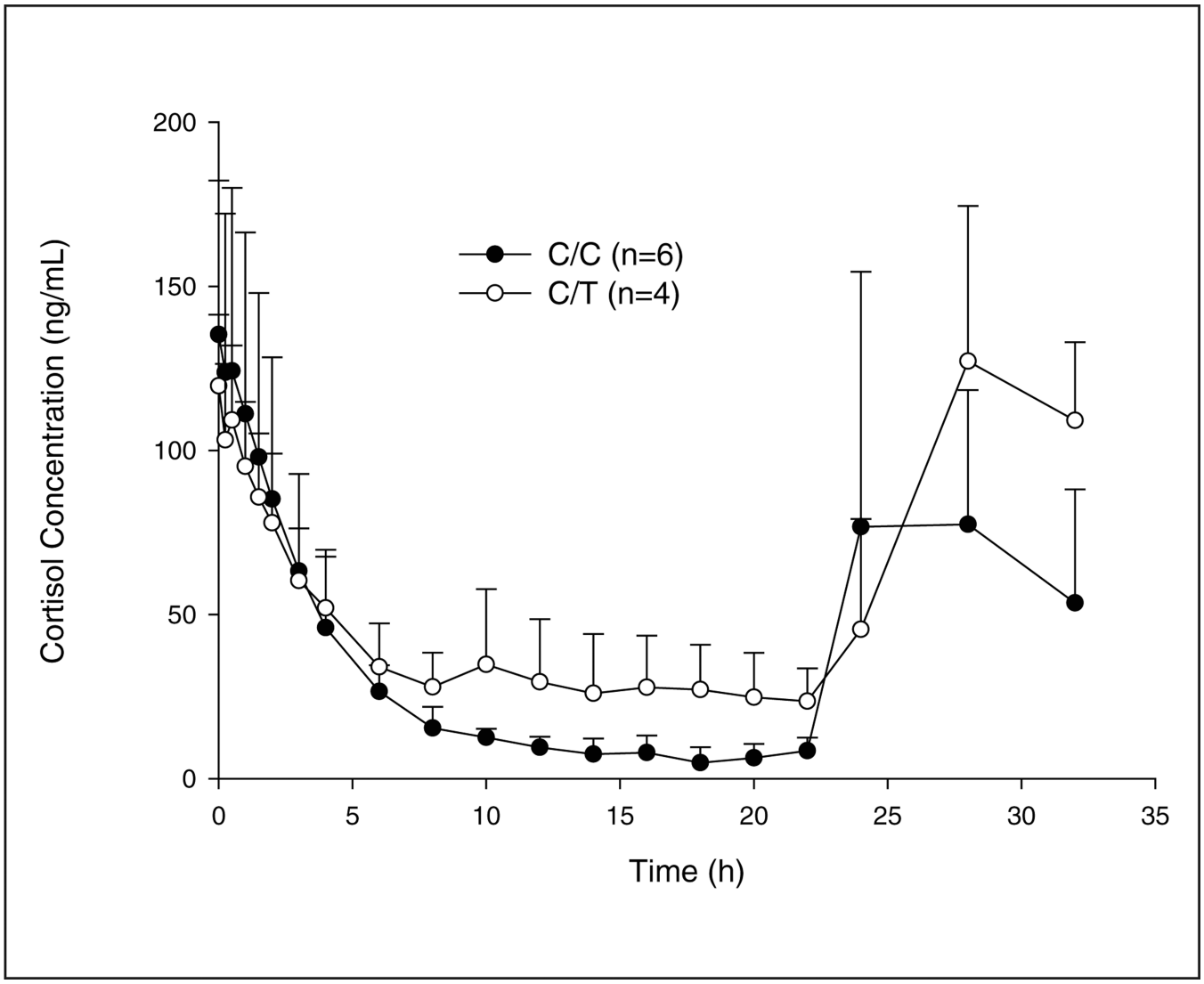

Figure 4.

Mean (±SD) plasma cortisol concentrations after the administration of methylprednisolone for subjects who are homozygous (C/C) and heterozygous (C/T) for the wild-type C314 allele.

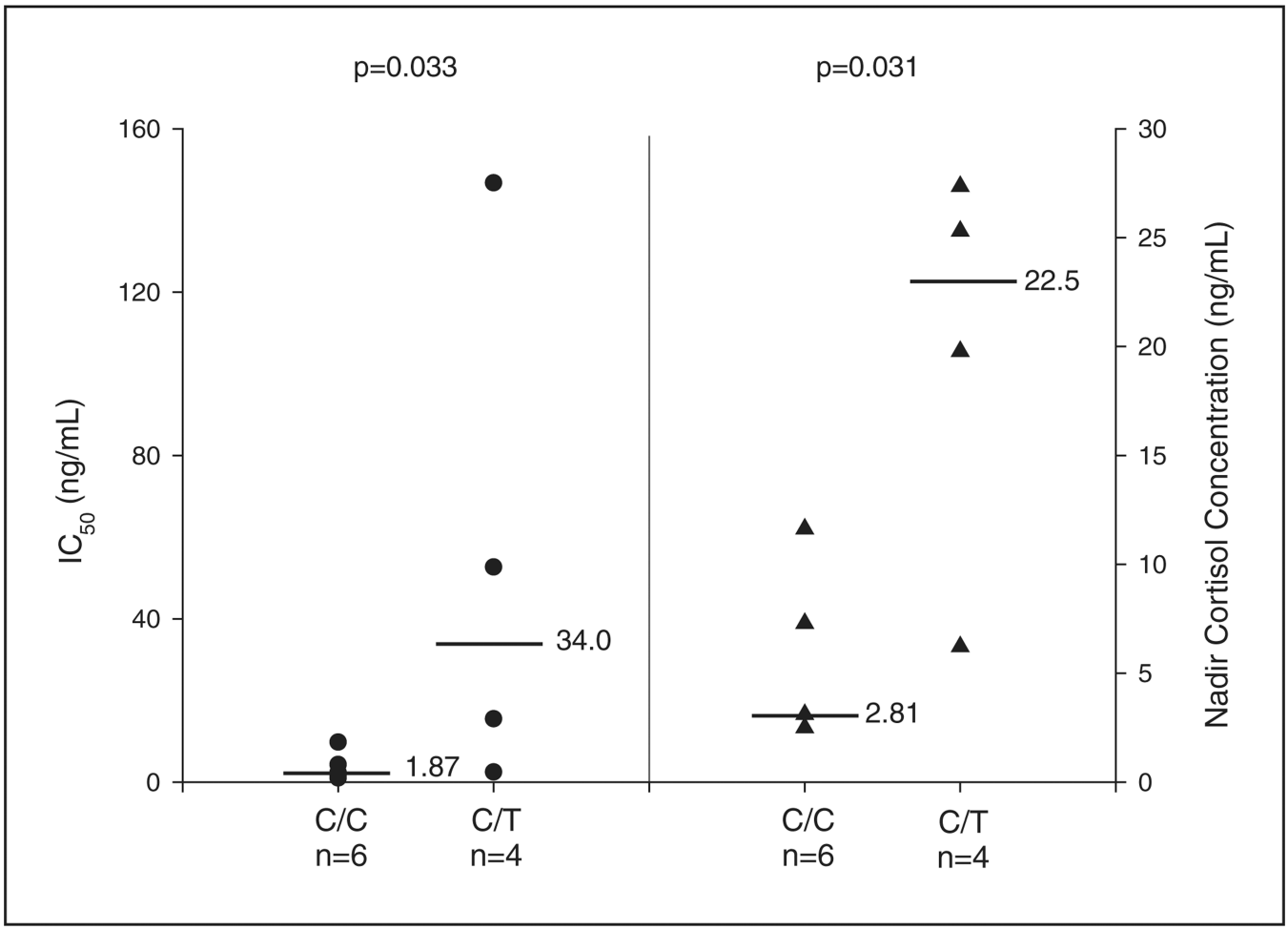

Figure 5.

Methylprednisolone concentrations causing 50% inhibition of the suppression of cortisol circadian secretion (IC_{50}) and nadir cortisol concentrations between subjects with C/C and C/T genotypes. Horizontal lines show median values.

Table I

Pharmacokinetic Parameters for Methylprednisolone

		HNMT C314T Genotype		
Parameter, units	**Definition**	**C/C Genotype (n = 6)**	**C/T Genotype (n = 4)**	***P***
k_{el}, h^{-1}	Elimination rate constant	0.237 (0.149– 0.320)	0.276 (0.228–0.300)	NS
Vd, L	Volume of distribution	95.1 (82.9–108)	85.1 (78.2–97.7)	NS
CL, L/h	Clearance	23.7 (13.6–29.1)	23.1 (21.7–25.0)	NS
T½, h	Half-life	2.93 (2.15–4.64)	2.51 (2.30–3.04)	NS
AUC, ng•h/mL	Area under curve	1745 (1378–3231)	1744 (1490–1950)	NS

Data are presented as median (range). Comparisons were made by Wilcoxon rank sum test. NS, not significant.

Table II

Pharmacodynamic Parameters for Cortisol Suppression

		HNMT C314T Genotype		
Parameter, units	**Definition**	**C/C Genotype (n = 6)**	**C/T Genotype (n = 4)**	***P***
k_{out}, h^{-1}	Loss constant	0.321 (0.225–0.350)	.370 (0.261–0.468)	NS
IC_{50}, ng/mL	Inhibition constant	1.87 (0.980–9.76)	34.0 (2.45–147)	.033
$AUEC_{SR}$	Area ratio	0.428 (0.177–0.820)	0.565 (0.362–0.769)	NS
ABEC, ng•h/mL	Area between response curve	1010 (242–1660)	776 (341–975)	NS

Data are presented as median (range). Comparisons were made by Wilcoxon rank sum test. NS, not significant.

Table III

Pharmacodynamic Parameters for Suppression of Whole Blood and Plasma Histamine Concentrations

		HNMT C314T Genotype		
Parameter, units	**Definition**	**C/C Genotype (n = 6)**	**C/T Genotype (n = 4)**	***P***
Whole blood histamine suppression				
$k_{r,}^{0}$ h^{-1}	Production constant	12.6 (7.55 to 27.4)	13.2 (6.00 to 21.9)	NS
k_H, h^{-1}	Loss constant	0.306 (0.196 to 0.34)	0.322 (0.272 to 0.350)	NS
IC_{50}, ng/mL	Inhibition constant	10.1 (2.75 to 224)	8.53 (5.20 to 22.3)	NS
$AUEC_{SR}$	Area ratio	0.409 (0.266 to 1.19)	0.408 (0.380 to 0.750)	NS
ABEC, ng•h/mL	Area between response curve	582 (−237 to 823)	411 (262 to 901)	NS
Plasma histamine suppression				
$AUEC_{SR}$	Area ratio	0.573 (0.317 to 0.700)	0.664 (0.639 to 0.720)	NS
ABEC, ng•h/mL	Area between response curve	3.04 (2.17 to 9.51)	3.17 (2.57 to 4.59)	NS

Data are presented as median (range). Comparisons were made by Wilcoxon rank sum test. NS, not significant.