Advancing Novel Anesthetics

Pharmacodynamic and Pharmacokinetic Studies of Cyclopropyl-methoxycarbonyl Metomidate in Dogs

Jason A. Campagna, M.D., Ph.D., Kevin Pojasek, Ph.D., David Grayzel, M.D., John Randle, Ph.D., Douglas E. Raines, M.D.

ABSTRACT

Background: Cyclopropyl-methoxycarbonyl metomidate (CPMM, also known as ABP-700) is a second-generation “soft” (i.e., metabolically labile) etomidate analogue. The purpose of this study was to characterize CPMM’s pharmacology in beagle dogs in preparation for potential first in human phase 1 clinical trials.

Methods: CPMM’s and etomidate’s hypnotic activity and duration of action were assessed using loss of righting reflex and anesthesia score assays in three or four dogs. Their pharmacokinetics were defined after single bolus administration and single bolus followed by 2-h infusion. Adrenocortical recovery times after single bolus followed by 2-h infusion of CPMM, propofol, etomidate, and vehicle were measured using an adrenocorticotropic hormone stimulation test.

Results: Compared with etomidate, CPMM was half as potent as a hypnotic (ED₅₀ approximately 0.8 mg/kg), was more rapidly metabolized, and had a shorter duration of sedative–hypnotic action. Recovery times after CPMM administration were also independent of infusion duration. After hypnotic infusion, adrenocorticotropic hormone–stimulated plasma cortisol concentrations were 4- to 27-fold higher in dogs that received CPMM versus etomidate. Adrenocortical recovery was faster in dogs after CPMM infusion versus etomidate infusion (half-time: 215 vs. 1,623 min, respectively). Adrenocortical responsiveness assessed 90 min after CPMM infusion was not significantly different from that after propofol infusion.

Conclusion: The studies in dogs confirm that CPMM has hypnotic and adrenocortical recovery profiles that are superior than those of etomidate, supporting the continued development of CPMM as a clinical sedative–hypnotic to be used as a single bolus and by continuous infusion to induce and maintain general anesthesia or procedural sedation. (Anesthesiology 2014; 121:1203-16)

ETOMIDATE entered into clinical practice in 1972 and because of its many favorable properties, it gained popularity as a single bolus agent to induce anesthesia and as a continuous infusion drug to maintain anesthesia or provide sedation.1–5 Unfortunately, recovery times from etomidate infusions are highly variable, and at hypnotic doses, etomidate also inhibits the cytochrome P450 enzyme 11β-hydroxylase, producing suppression of adrenocortical steroid synthesis that persists long after the hypnotic effects dissipate.2–10 The potential serious consequences of such potent and persistent adrenocortical suppression led clinicians to abandon the use of etomidate infusions and, while still controversial, have also raised concerns about the administration of a single etomidate bolus for anesthetic induction.11–18

Several years ago, we developed the hypothesis that ultra–short-acting analogues of etomidate could be designed that would retain many desirable properties of etomidate, but afford faster hypnotic recovery and remove any clinically significant adrenocortical suppression.19 We established proof-of-principle by synthesizing the prototypical “soft” (i.e., metabolically labile) etomidate analogue methoxycarbonyl etomidate but found that its pharmacological properties were not suitable for clinical development.19–22 Subsequent efforts at rational optimization of methoxycarbonyl etomidate are described in this report.

Submitted for publication February 27, 2014. Accepted for publication July 28, 2014. From the Medicines Company, Inc., Parsippany, New Jersey (J.A.C.); Annovation BioPharma, Inc., Cambridge, Massachusetts (K.P., D.G., J.R.); and Department of Anesthesia, Critical Care, and Pain Medicine, Massachusetts General Hospital, Boston, Massachusetts (D.E.R.).

Copyright © 2014, the American Society of Anesthesiologists, Inc. Lippincott Williams & Wilkins. Anesthesiology 2014; 121:1203-16
etomidate’s pharmacology led to the synthesis and study of more than a dozen new analogues, of which cyclopropylmethoxycarbonyl metomidate (CPMM, also known as ABP-700) exhibited the most promising pharmacology in rats (fig. 1) and became the lead candidate for advancement to human trials.23–25

Although rats are commonly used as the initial animal model to assess the pharmacology of new therapeutic agents, studies in larger nonrodent species provide additional insights into the pharmacology, safety, and efficacy of novel drug candidates.26,27 Therefore, global regulatory authorities require studies in a nonrodent species as part of any investigational new drug application intended to advance a new chemical entity into human clinical testing.28,29 In this article, we report the results of studies conducted to define the pharmacology of CPMM in dogs. Our goal in this work was to characterize the pharmacodynamic and pharmacokinetic properties of CPMM with the purpose of advancing knowledge about this potential new anesthetic agent.

Materials and Methods

The methodology of the in vivo laboratory studies was dictated in part by international guidelines established by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use and, as a result, may differ from those routinely encountered for research studies of this type.

Animals

All animal studies were conducted in accordance with the Final Rules of the Animal Welfare Act regulations, the Public Health Service Policy on Humane Care and Use of Laboratory Animals from the Office of Laboratory Animal Welfare of the National Institutes of Health, and the Guide for the Care and Use of Laboratory Animals from the National Research Council. Animals (n = 4 dogs) were purchased from Marshall BioResources (North Rose, NY). Studies were performed at VivoPath, Inc. (Worcester, MA) and their Animal Care and Use Committee approved all protocols before initiation of any study. Young male beagle dog littersmates weighing between 5.2 and 9.2 kg at the time of study were used. All four animals participated, on different occasions, in all studies except the single bolus pharmacokinetic studies in which only three dogs were used. For single-bolus hypnotic potency and duration studies, animals were dosed up to twice each day. For all other studies (i.e., behavioral recovery, pharmacokinetic, and adrenocortical suppression studies), animals were given at least 6 days to recover between dosing. Animals were housed with 12-h light–dark cycles (lights on at 7:00 AM and off at 7:00 PM). Test drug and vehicle were administered through a 22-gauge IV catheter located in a saphenous vein.

Sources and Formulation of Hypnotic Drugs

Cyclopropyl-methoxycarbonyl metomidate was synthesized at Aberjona Laboratories (Beverly, MA) using the approach described previously and formulated (8 mg/ml) as an aqueous solution with 20% sulfobutylether-β-cyclodextrin (adjusted to pH 7.4).23 The formulated drug was stored frozen at −20°C with ongoing stability testing of representative batches demonstrating no significant degradation over time. Etomidate was purchased from BaChem (Torrance, CA) and formulated (2 mg/ml) as an aqueous solution in 35% propylene glycol. CPMM and etomidate solutions were sterile filtered through a 0.2-μm polysulfone filter before dosing. Propofol (10 mg/ml) was purchased from Abbott Laboratories (Abbott Park, IL) formulated in the standard emulsion.

Hypnotic Potency and Duration of Hypnotic Action

By using a loss of righting reflexes (LoRR) assay, hypnotic potencies and durations of hypnotic action were assessed in dogs. The desired dose of hypnotic (CPMM or etomidate) was rapidly (<5 s) injected intravenously followed by a normal saline flush. Immediately after injection, dogs were turned onto their sides. A dog was judged to have LoRR if it failed to immediately right itself (onto all four paws) after drug administration. A stopwatch was used to measure the duration of LoRR, which was defined as the time from drug injection until spontaneous righting.

Dosing of Hypnotic Drugs for Behavioral, Pharmacokinetic, and Adrenocortical Recovery Studies

In all studies, the goal was to attain an intermediate depth of hypnosis/anesthesia, which we defined as an anesthesia score of 3 (table 1). For all studies, this was achieved with a rapid (<5 s) bolus dose of 3 to 4 mg/kg for CPMM, 2 mg/kg for etomidate, or 5 to 6 mg/kg for propofol. Because of its low therapeutic index,30 propofol boluses were given at 1 mg/kg increments every 20 s until an anesthesia score of 3 was reached. For 2-h maintenance studies, this bolus was followed by a 2-h continuous infusion. During these 2-h infusions, the infusion rates were adjusted between 0.5 to

![Fig. 1. Chemical structures of etomidate and cyclopropylmethoxycarbonyl metomidate (CPMM) and their respective primary metabolites.](image-url)
1.0 mg kg⁻¹ min⁻¹ for CPMM, 0.1 to 0.15 mg kg⁻¹ min⁻¹ for etomidate, and 0.4 to 0.5 mg kg⁻¹ min⁻¹ for propofol to maintain an anesthesia score of 3. For 2-h infusion studies comparing recovery after CPMM versus etomidate, behavioral, pharmacokinetic, and adrenocortical studies were done simultaneously in each individual dog. During 2-h infusion studies, dogs could receive midazolam (0.1 to 0.2 mg/kg IV bolus per dose) as required to suppress involuntary movements (i.e., myoclonus). In pilot experiments, such dosing had no discernable sedative effect but in our studies effectively reduced the myoclonus. During hypnotic infusions and until anesthetic emergence, supplemental oxygen was supplied to dogs by facemask.

**Behavioral Recovery after Hypnotic Bolus or Hypnotic Bolus Followed by Continuous Infusion: Assessment Using an Anesthesia Scale**

Anesthetic depth was quantified using a subjective numerical anesthetic scale similar to that used in the study reported by Silva et al. (table 1). This scale ranged from a score of 0 (fully awake and alert) to a score of 4 (deep anesthesia). In some experiments, scores were judged to fall between scores of 0 and 1, 1 and 2, or 2 and 3 during recovery. These were recorded as scores of 0.5, 1.5, or 2.5, respectively, but were not included in our analyses because not all dogs were judged to have attained these intermediate anesthetic depths and these scores were not associated with specific behavioral endpoints. For single-bolus-only studies, anesthetic depth was assessed and the anesthetic score recorded at times 0 (i.e., immediately before drug administration), 9, 15, and 30 s after hypnotic administration, and every 30 s thereafter until dogs fully recovered. For studies using a single bolus followed by 2-h hypnotic maintenance infusion, anesthetic depth was intermittently assessed and the anesthetic score recorded until dogs fully recovered.

**Handling of Blood Samples for Pharmacokinetic Studies**

At the desired time points after hypnotic administration (either single bolus alone or single bolus followed by 2-h infusion), blood samples (approximately 1 ml) were drawn from an IV catheter placed in the cephalic vein and transferred into prechilled collection tubes containing EDTA. Esterase activity in the collected blood was immediately inactivated by the addition of acetonitrile. Blood samples were maintained chilled (0° to 4°C), centrifuged within 30 min, and the plasma stored at −80°C until analyzed. Plasma concentrations of CPMM, etomidate, and their respective primary metabolite were quantified by Nexerca, Inc. (Woburn, MA) using a high-performance liquid chromatography mass spectrometry assay coupled with tandem mass spectrometry. Samples (20 μl) were injected onto a Luna C8 R (5 μm, 2.0 × 50 mm) column using a CTC Analytics HTS Pal Autosampler (Zwingen, Switzerland) and an Agilent binary 1100 LC pump (Santa Clara, CA). The high-performance liquid chromatography mobile phases were (phase A) 0.1% formic acid in water (v/v) and (phase B) 0.1% formic acid in 90/10 acetonitrile/water (v/v). The gradient ran from 25 to 90% phase B in 2.6 min and the flow rate was 0.3 ml/min. The total running time per sample was 4 min. The analytes were detected with an API 5000 triple quadrupole mass spectrometer (AB SCIEX, Framingham, MA). The intensities of CPMM, etomidate, acid metabolites, and internal standard were determined by integration of extracted ion-peak areas using Analyst 1.5.1 software (AB SCIEX). Calibration curves were prepared by plotting the analyte to internal standard peak area ratio versus concentration. The model for the calibration curves was linear with (1/x²) weighting. The CPMM and etomidate calibration curves were linear between 0.1 to 250 ng/ml with 75% or more of the standards (two replicates of eight concentrations) and 67% or more of the QCs (three replicates of three concentrations) with ±15% of the spiked concentration. The CPMM metabolite and etomidate metabolite calibration curves were linear between 5 to 1,000 ng/ml with 75% or more of the standards (two replicates of seven concentrations) and 67% or more of the QCs (three replicates of three concentrations) with ±15% of the spiked concentration.

**Pharmacokinetic Modeling**

Pharmacokinetic parameters were derived by noncompartmental methods using Phoenix WinNonlin Version 6.3 (Pharsight Corp., Cary, NC). The following parameters for

---

**Table 1. Behavioral Assessment and Associated Anesthesia Scale**

<table>
<thead>
<tr>
<th>Anesthesia Depth</th>
<th>Behavioral Signs</th>
<th>Anesthesia Scores*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awake</td>
<td>Fully awake and alert</td>
<td>0</td>
</tr>
<tr>
<td>Sedated</td>
<td>Mildly impaired but responsive to stimulation and with righting reflexes present</td>
<td>1</td>
</tr>
<tr>
<td>Shallow anesthesia</td>
<td>Righting reflexes lost but responds to stimulation (e.g., presence of oximeter probe on the tongue)</td>
<td>2</td>
</tr>
<tr>
<td>Medium anesthesia</td>
<td>Responds only to painful stimulation (e.g., paw pinch). No response to oximeter probe on the tongue</td>
<td>3</td>
</tr>
<tr>
<td>Deep anesthesia</td>
<td>Unresponsive to painful stimuli, reduced respirations, oxygen desaturation</td>
<td>4</td>
</tr>
</tbody>
</table>

* Scores of 0.5, 1.5, or 2.5 were sometimes recorded during recovery when behavior was judged to fall between scores 0 and 1, 1 and 2, or 2 and 3, respectively.
each analyte were derived, where appropriate, from individual plasma concentration–time profiles in individual dogs:

- $C_0$: the plasma concentration after IV bolus administration at $t = 0$ min determined by WinNonlin by back-extrapolation from the initial concentration–time points.
- $C_{\text{max}}$: the maximum observed plasma concentration.
- $t_{\text{max}}$: the time of occurrence of $C_{\text{max}}$.
- $\lambda_z$: the apparent terminal elimination rate constant, calculated as the absolute value of the slope of the terminal phase of the natural log-transformed plasma concentration–versus-time curve.
- $t_{1/2}$: the apparent terminal half-life, calculated as $\ln 2/\lambda_z$.
- AUC: the area under the plasma concentration-versus-time curve from time zero to infinity, calculated from $AUC_{\text{last}} + C_t/\lambda_z$, where $C_t$ is the concentration at the last measurable time point.
- $V_z$: the apparent volume of distribution in the terminal phase, calculated as $\text{dose}/(\lambda_z \times AUC)$ for the dosed drug.
- $V_{\text{ss}}$: the apparent volume of distribution at steady state, calculated as $\text{dose}/AUC_{\text{tr}}$ for the dosed drug.
- $CL$: systemic clearance, calculated as $\text{dose}/AUC_{\text{tr}}$ for the dosed drug.

Nominal sampling times were used for all calculations of pharmacokinetic parameters. Plasma concentrations below the limit of quantitation of the assay were taken as zero if they occurred before the first measurable time point and as missing if they occurred after the last measurable time point or in between two measurable time points. For the estimation of $\lambda_z$ (the apparent terminal elimination rate constant) and corresponding $t_{1/2}$ (the apparent terminal half-life), calculated as $\ln 2/\lambda_z$ values, three or more points were required within the terminal phase ($C_{\text{max}}$ could not be included). In addition, adjusted $R^2$ for $\lambda_z$ (goodness-of-fit statistic for the terminal elimination phase adjusted for number of points used) was required to be greater than 0.85.

Pharmacodynamic Modeling
For CPMM and etomidate, the relationship between plasma hypnotic concentrations and anesthesia scores upon bolus injection were fit to a sigmoid $E_{\text{max}}$ model (equation 1)$^{33}$:

$$E(t) = E_{\text{max}} X \frac{C_f(t)^\gamma}{C_f(t)^\gamma + EC_{\text{AS}}^\gamma}$$

where: $E(t)$ is the anesthesia score at time $t$; $E_{\text{max}}$ is the maximum anesthesia score, which is a fixed value of 4 on our scale; $C_f(t)$ is the plasma hypnotic concentration at time $t$; $EC_{\text{AS}}$ is the plasma concentration when anesthesia scores are 25, 50, and 75% of the maximal anesthesia score and correspond to anesthesia scores of 1, 2, and 3, respectively; and $\gamma$ is the slope parameter which reflects the steepness of the concentration–response relation.

Adrenocortical Recovery after Hypnotic Infusion
Anesthesia was induced and maintained for 2 h at an anesthesia score of 3 with the desired hypnotic agent (CPMM, propofol, or etomidate) as described above under Dosing of Hypnotic Drugs for Behavioral, Pharmacokinetic, and Adrenocortical Recovery Studies. To minimize baseline cortisol concentrations and increase the sensitivity of the assay, endogenous adrenocorticotrophic hormone (ACTH) production was suppressed by intravenously administering dexamethasone (0.10 mg/kg) at the start of hypnotic infusions ($t = 0$ min) and then every 2 h during the study ($t = 120$ and 240 min). For studies comparing adrenocortical responsiveness after infusion of CPMM and etomidate, a control group that received a bolus followed by infusion of CPMM's vehicle was also studied. At the desired times after the 2-h hypnotic or vehicle infusion ended, ACTH $1–24$ (250 μg) was administered intravenously at 90-min intervals and blood samples were drawn every 30 min. Plasma cortisol concentrations in sampled blood were quantified using an enzyme-linked immunosorbent assay (Nextcea, Inc.).

Statistical Analysis
Unless indicated otherwise, data points are reported as mean ± SD and fitted parameters are reported as mean with 95% CIs. For values defined by the ratio of two experimental values ($i.e.$, the normalized cortisol concentration), the reported SD was determined by error propagation. Anesthetic recovery times from an anesthesia score of 3 (to lower scores) after hypnotic administration were compared between the two hypnotic groups using a two-way ANOVA with a Bonferroni posttest to correct for multiple comparisons. The factors were anesthesia score and hypnotic group. Plasma cortisol concentrations after hypnotic or vehicle administration were compared using a two-way ANOVA with a Tukey multiple comparisons test. Comparisons of in vivo adrenocortical inhibitory potencies were made using an extra-sum-of-squares $F$ test. Statistical analyses and curve fits were done using Prism v5.0 for the Macintosh (GraphPad Software, Inc., La Jolla, CA) or Igor Pro 6.1 (Wavemetrics, Lake Oswego, OR).

Results
Hypnotic Activity of CPMM and Etomidate
Our first studies in dogs were designed to confirm CPMM’s hypnotic activity in a large nonrodent species using an LoRR assay and to establish potency for subsequent behavioral recovery experiments. CPMM was administered as an IV bolus and rapidly (<15 s) induced LoRR in all dogs at doses 1 mg/kg or greater, whereas lower doses ($i.e.$, 0.25 or 0.5 mg/kg) failed to induce LoRR in any dog (fig. 2A). For dogs that had LoRR, the duration of LoRR increased approximately linearly with the logarithm of the dose from an average value of 1.8 ± 0.6 min (at 1 mg/kg, n = 3 dogs) to 10.9 ± 0.7 min (at 12 mg/kg, n = 4 dogs; fig. 2B). The X-intercept and slope of...
this relation were 0.88 mg/kg (95% CI, 0.50 to 1.27 mg/kg) and 9.9 (95% CI, 9.4 to 12.5), respectively.\textsuperscript{34,35} For comparison, we also assessed etomidate’s hypnotic activity in dogs with bolus doses ranging from 0.5 to 1.9 mg/kg. Over this dose range, etomidate rapidly produced LoRR in all dogs (fig. 2A). The duration of LoRR produced by etomidate also increased approximately linearly with the logarithm of the dose from 2.7 ± 0.8 min (at 0.5 mg/kg, n = 3 dogs) to 17 ± 5 min (at 1.9 mg/kg, n = 3 dogs; fig. 2B). For etomidate, the X-intercept and slope of this relation were 0.37 mg/kg (95% CI, 0.18 to 0.52 mg/kg) and 24 (95% CI, 15 to 34), respectively, indicating that etomidate was approximately twice as potent as CPMM in dogs and cleared from the brain significantly more slowly.

Behavioral Recovery after Administration of CPMM and Etomidate

We then quantified the rate at which dogs transitioned from a medium depth of anesthesia to full hypnotic recovery after CPMM administration (table 1). As with the LoRR studies, we used etomidate (at an approximately equihypnotic dose) as a comparator. For individual dogs, figure 3A plots the anesthesia score as a function of time after single bolus administration of either CPMM or etomidate. Figure 3B graphs the mean times (±SD) required for dogs to recover (from an anesthesia score of 3) to the indicated anesthesia scores after receiving the hypnotic bolus. The data show that the times required for dogs to recover from an anesthesia score of 3 to all lower scores were significantly shorter after bolus administration of CPMM as compared with etomidate.

For individual dogs, figure 4A plots the anesthesia score as a function of time as dogs recovered after receiving a single bolus dose of either CPMM or etomidate followed by a 2-h infusion (using the same hypnotic) that maintains an anesthesia score of 3. Figure 4B graphs the mean time (±SD) required for dogs to recover (from an anesthesia score of 3) to the indicated anesthesia score after the 2-h infusion was completed. It shows that the times required for dogs to recover from an anesthesia score of 3 to all lower scores was shorter after administration of CPMM versus etomidate, with statistical significance achieved at scores of 1 and 0. Our analysis also showed that there was no difference in the time required to recover to anesthesia scores of 2, 1, or 0 after administering a single bolus of CPMM as compared with that after administering a single bolus followed by a 2-h infusion of CPMM (4.4 ± 1.3 min, 6.0 ± 1.1 min, or 8 ± 3 min for bolus only vs. 4.3 ± 1.2 min, 5.5 ± 0.7 min, or 11 ± 3 min for bolus followed by a 2-h infusion). Thus, hypnotic recovery times after CPMM administration were independent of infusion duration.

During administration of CPMM and etomidate, myoclonus was observed in some dogs. The myoclonic movements were modest in intensity and consisted primarily of tremors or twitching in the torso and neck. After 2-h infusions, myoclonus persisted long (up to approximately an hour) into the postinfusion period after etomidate infusions but ceased within several minutes after terminating CPMM infusions. During CPMM infusion, all the four dogs had myoclonus and were given midazolam (a single dose of 0.2 mg/kg to two dogs and 0.4 mg/kg in divided doses to two dogs) to attenuate the myoclonus. Similarly, during etomidate infusion, two dogs had myoclonus and were given midazolam (a single dose of 0.2 mg/kg to one dog and 0.3 mg/kg in divided doses to another dog).

In Vivo Pharmacokinetics of CPMM and Etomidate: Single Bolus and Infusion Studies

We also measured the time-dependent change in the plasma concentration of parent hypnotic and metabolite after administering CPMM or etomidate to dogs as either a single bolus or a 2-h infusion of the hypnotic. The pharmacokinetics were best described using a two-compartment model with a linear first-order elimination phase. The analysis showed that the pharmacokinetics of CPMM and etomidate were similar, with the exception that the plasma concentration of the metabolite of CPMM was higher than that of etomidate. This finding suggests that CPMM is metabolized more rapidly than etomidate in dogs.
IV bolus or a single bolus followed by a 2-h infusion (fig. 5). Figure 5A plots the plasma CPMM and CPMM metabolite concentrations as a function of time after bolus administration of CPMM (4 mg/kg, n = 3 dogs). The plasma CPMM concentration in the initial blood sample, obtained 30 s after CPMM administration, was 4,130 ± 640 ng/ml. Plasma CPMM concentrations in blood samples drawn 10 min after CPMM administration were 330 ± 41 ng/ml (i.e., after full hypnotic recovery) and decreased by one order of magnitude during the subsequent 115 min of the study. In the final blood samples drawn 2 h after CPMM administration, plasma CPMM concentrations had decreased by four orders of magnitude from those measured in the initial blood sample. The plasma CPMM concentration range during anesthetic recovery (i.e., the CPMM concentration present 4.4 to 8 min after bolus administration, see fig. 3B) was approximately 500 ng/ml. Coincident with the time-dependent reduction in plasma CPMM concentrations, we measured a time-dependent change in plasma CPMM metabolite concentrations. The peak metabolite concentration (13,750 ± 2,570 ng/ml) was measured in samples drawn 5 min after bolus CPMM administration. This concentration decreased by one order of magnitude during the subsequent 115 min of the study.

Figure 5B plots the plasma etomidate and etomidate metabolite concentrations as a function of time after bolus administration of etomidate (2 mg/kg, n = 3 dogs) to dogs. The plasma etomidate concentration in the initial blood sample, which was obtained 30 s after etomidate administration, was 9,080 ± 1,390 ng/ml. Plasma etomidate concentrations in blood samples drawn 10 min after etomidate administration were 2,230 ± 448 ng/ml (i.e., 25% of that present in the initial blood sample). In the final blood samples drawn 120 min after etomidate administration, the plasma etomidate concentration was 1.5 orders of magnitude lower than that measured in the initial samples. The plasma etomidate concentration range associated with anesthetic recovery
(i.e., the etomidate concentration present 9.0 to 13.8 min after bolus administration, see fig. 3B) was approximately 1,000 ng/ml. We also measured a time-dependent change in plasma etomidate metabolite concentrations. This concentration reached a peak value of 2,330 ± 587 ng/ml in blood samples drawn 10 min after etomidate administration before steadily decreasing to 708 ± 75 ng/ml in our final blood samples drawn 120 min after etomidate administration.

Figure 5C shows the plasma CPMM and CPMM metabolite concentrations in dogs as a function of time after administering a single bolus followed by a 2-h continuous infusion of CPMM. Each data point in the graph is the mean ± SD (n = 3 or 4 dogs for each data point). The plasma CPMM concentration in blood drawn immediately before the infusion ended was 3,310 ± 740 ng/ml. In the next blood sample (which was drawn 30 min after the infusion ended), the plasma CPMM concentration was 103 ± 56 ng/ml. This concentration is just 3% of that measured immediately before the infusion ended and well below that which we associate with full hypnotic recovery. After this rapid initial decrease, plasma CPMM concentrations in subsequent blood samples decreased more slowly (e.g., by only 22% to 81 ± 27 ng/ml during the next 30 min) before reaching 15 ± 12 ng/ml in blood samples drawn 300 min after the infusion ended. The plasma concentration of CPMM metabolite in blood drawn immediately before the infusion ended was 36,240 ± 6,970 ng/ml. This concentration decreased by two orders of magnitude to 340 ± 200 ng/ml in our final blood samples drawn 300 min later. Figure 5D shows the plasma etomidate and etomidate metabolite concentrations as a function of time after administering etomidate as a single bolus followed by a 2-h infusion (n = 4 dogs). The plasma etomidate concentration in blood drawn immediately before the infusion ended was 2,050 ± 1,090 ng/ml. In the next blood sample drawn 30 min later, the plasma etomidate concentration decreased to 480 ± 340 ng/ml which is 23% of that measured immediately before the infusion ended and within the hypnotic range. Plasma etomidate concentrations in subsequent blood samples progressively decreased reaching 37 ± 24 ng/ml in blood samples drawn 300 min after the infusion ended. The plasma concentration of etomidate metabolite was highest in blood drawn immediately before the infusion ended (1,680 ± 250 ng/ml) and decreased by more than an order of magnitude in blood samples drawn 300 min later.
The Pharmacology of CPMM in Dogs

We modeled the time-dependent change in the venous concentrations of CPMM and etomidate (and their respective metabolites) in each dog using noncompartmental analysis. The mean ± SD values for the derived parameters are shown in tables 2 and 3 for single bolus and single bolus followed by 2-h infusion studies, respectively. After the single bolus administration, the concentrations of both CPMM and etomidate declined in a biexponential manner. The terminal elimination half-life of CPMM was 27% that of etomidate with values of 16.1 ± 2.99 min and 60.1 ± 10.4 min, respectively (P = 0.0195). Similarly, clearance of CPMM was 14 times higher than that of etomidate with values of 211 ± 26 ml kg⁻¹ min⁻¹ and 14.7 ± 2.78 ml kg⁻¹ min⁻¹, respectively (P = 0.0062). In the case of CPMM (but not etomidate), clearance exceeded the total hepatic blood flow in dogs (by an order of magnitude), indicating that hydrolysis of CPMM to its carboxylic acid metabolite was primarily extrahepatic. CPMM’s apparent volume of distribution at steady state (1,880 ± 569 ml/kg) was not only significantly larger than that of etomidate, it was three times greater than the total body water volume in dogs (600 ml/kg). This suggests significant CPMM distribution into tissues. The area under the plasma concentration-time curve also significantly differed between CPMM and etomidate with respective values of 19,200 ± 2,590 min ng ml⁻¹ and 139,000 ± 26,300 min ng ml⁻¹ (P = 0.0014). After single bolus followed by 2-h infusion, the difference in terminal elimination half-lives of the two drugs was not significantly different with values of 96.8 ± 38.5 min and 88.1 ± 14.1 min for CPMM and etomidate, respectively (P = 0.6788).

For a single representative dog, figure 6, A and B, plots the time-dependent change in plasma hypnotic concentrations and anesthesia scores upon bolus administration of 4 mg/kg of CPMM (fig. 6A) or 2 mg/kg of etomidate (fig. 6B). For both CPMM and etomidate, anesthesia scores and plasma concentrations decreased over time after bolus administration. Figure 6, C and D, plots for all dogs the relation between the anesthesia score and the plasma hypnotic concentration after bolus administration of 4 mg/kg of CPMM (fig. 6C) or 2 mg/kg of etomidate (fig. 6D). Fits of these data to equation 1 yielded the venous concentrations of CPMM and etomidate associated with anesthesia scores of 1, 2, or 3 (table 4).

### Adrenocortical Recovery after Prolonged Administration of CPMM: Comparisons with Propofol and Etomidate

The adrenocortical recovery profile of CPMM was assessed and compared with that of the commonly infused hypnotic propofol by inducing and maintaining dogs under anesthesia for 2 h (at an anesthesia score of 3) with either CPMM or propofol and then measuring ACTH₁–24-stimulated plasma cortisol concentrations in serially drawn blood samples (n = 4 dogs per group). During these studies, myoclonus was observed with both CPMM and propofol. During CPMM infusion, all four dogs had myoclonus and were given midazolam (0.4 mg/kg in divided doses to two dogs and 0.6 mg/kg in divided doses to two dogs) to eliminate myoclonus. During propofol infusion, two dogs had myoclonus and were given midazolam (0.2 mg/kg to each dog). Figure 7 plots the plasma cortisol concentrations at the indicated times after hypnotic infusion termination and upon stimulation with ACTH₁–24. In both hypnotic groups, plasma cortisol concentrations increased after ACTH₁–24 administration, and adrenal responsiveness to ACTH₁–24 was virtually identical between dogs that received CPMM and those that received propofol.

We also compared the adrenocortical response recovery profile after administration of CPMM to that after administration of etomidate as the latter is known to produce

### Table 2. Pharmacokinetic Parameters upon Bolus Administration (n = 3 Dogs)

<table>
<thead>
<tr>
<th></th>
<th>C₀ (ng/ml)</th>
<th>tₘax (min)</th>
<th>AUC (min-ng ml⁻¹)</th>
<th>CL (ml·kg⁻¹·min⁻¹)</th>
<th>Vss (ml/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPMM</td>
<td>5,180 ± 1,120</td>
<td>16.1 ± 2.99*</td>
<td>19,200 ± 2,590*</td>
<td>211 ± 26**</td>
<td>1,880 ± 569</td>
</tr>
<tr>
<td>Etomidate</td>
<td>10,700 ± 1,440</td>
<td>60.1 ± 10.4</td>
<td>139,000 ± 26,300</td>
<td>14.7 ± 2.78</td>
<td>874 ± 172</td>
</tr>
</tbody>
</table>

CPMM vs. etomidate or CPMM metabolite vs. etomidate metabolite: * P < 0.05; ** P < 0.01.

### Table 3. Pharmacokinetic Parameters after Bolus Followed by 2-h Infusion (n = 4 Dogs)

<table>
<thead>
<tr>
<th></th>
<th>Cmax (ng/ml)</th>
<th>tₘax (min)</th>
<th>AUC (min-ng ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPMM</td>
<td>1,530 ± 1,060</td>
<td>11.7 ± 7.64</td>
<td>30.3 ± 2.09*</td>
</tr>
<tr>
<td>Etomidate</td>
<td>2,370 ± 347</td>
<td>18.3 ± 18.9</td>
<td>61.4 ± 9.13</td>
</tr>
</tbody>
</table>

CPMM vs. etomidate or CPMM metabolite vs. etomidate metabolite: P < 0.01.
In these studies, we induced and maintained dogs at an anesthesia score of 3 for 2 h with either CPMM or etomidate (n = 4 dogs per group). We also included a vehicle group as a control that received CPMM’s vehicle only.

Figure 8A plots the plasma cortisol concentration in dogs at the indicated times after infusion with CPMM, etomidate, or vehicle and upon stimulation with ACTH1–24. In both hypnotic groups, plasma cortisol concentrations were lower than vehicle-treated animals at the first postinfusion sample drawn 30 min after infusion termination (and administration of the first dose of ACTH1–24).

The CPMM-treated animals exhibited a normal adrenal response to the second ACTH challenge (180 min postinfusion) when compared with the vehicle control, whereas the etomidate-treated animals continued to show adrenal hyporesponsiveness. Plasma cortisol concentrations in the etomidate-treated group remained significantly lower than those in the vehicle group throughout the duration of the study (>300 min), whereas plasma cortisol concentrations in the CPMM group were not significantly different from controls for all time points longer than 120 min after infusion termination. At equivalent postinfusion time points, plasma cortisol concentrations were 4- to 27-fold higher.

Table 4. Pharmacodynamic Parameters upon Bolus Administration (n = 3 Dogs)

<table>
<thead>
<tr>
<th></th>
<th>EC_{anesthesia score 1} (95% CI), ng/ml</th>
<th>EC_{anesthesia score 2} (95% CI), ng/ml</th>
<th>EC_{anesthesia score 3} (95% CI), ng/ml</th>
<th>( \gamma ) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPMM</td>
<td>520 (297–911)</td>
<td>1,206 (894–1,627)</td>
<td>2,798 (1,840–4,256)</td>
<td>1.3 (0.7–1.9)</td>
</tr>
<tr>
<td>Etomidate</td>
<td>1,607 (1,026–2,517)</td>
<td>3,092 (2,425–3,944)</td>
<td>5,952 (4,392–8,066)</td>
<td>1.7 (0.9–2.4)</td>
</tr>
</tbody>
</table>

CPMM = Cyclopropyl-methoxycarbonyl metomidate; EC_{anesthesia score 1} = venous plasma concentration when the anesthesia score is 1; EC_{anesthesia score 2} = venous plasma concentration when the anesthesia score is 2; EC_{anesthesia score 3} = venous plasma concentration when the anesthesia score is 3; \( \gamma \) = slope parameter.
in blood drawn from dogs in the CPMM group compared with those in the etomidate group. For each time point after infusion, the inset in figure 8A plots the plasma cortisol concentration in each hypnotic group normalized to that in the vehicle control group. It shows that after the hypnotic infusions ended, plasma cortisol concentrations in blood drawn from both hypnotic groups recovered steadily over time toward those of the vehicle control group. The half-time for that recovery was calculated from that data to be 215 min (95% CI, 152 to 362 min) in the CPMM group and 1,623 min (95% CI, 1,041 to 3,763 min) in the etomidate group ($P < 0.0001$).

Together, the pharmacokinetic (fig. 5, C and D) and adrenocortical data (fig. 8A and inset) allow us to broadly assess the relation between the plasma hypnotic concentration (determined in the former) and adrenocortical responsiveness to ACTH$_{1-24}$ stimulation (determined in the latter). Figure 8B plots that relation and shows that when plasma concentrations of CPMM or etomidate were high soon after infusions ended, adrenocortical responsiveness (as reflected by normalized plasma cortisol concentrations) was low. From these data, we estimated in vivo half-inhibitory concentrations of 27 ng/ml (95% CI, 22 to 32 ng/ml) for CPMM and 16 ng/ml (95% CI, 8.5 to 30 ng/ml) for etomidate ($P = 0.0234$). In the case of etomidate, the estimated half-inhibitory concentration for adrenal steroidogenesis correlates well with the IC$_{50}$ of this drug defined in previous reports.$^{31,42}$

**Discussion**

These are the first studies to define the pharmacology of the soft etomidate analogue CPMM in a large nonrodent species. They show that in beagle dogs, CPMM is a rapidly metabolized and ultra–short-acting hypnotic agent with a potency that is approximately half that of etomidate. Unlike etomidate however, CPMM has a hypnotic recovery profile that is independent of infusion duration with full recovery from a medium depth of anesthesia occurring as quickly after single bolus administration as after single bolus followed by a 2-h infusion. In addition, we found that CPMM and propofol have equivalent adrenocortical recovery profiles and that ACTH$_{1-24}$-stimulated cortisol concentrations increase much more rapidly after CPMM infusion than after etomidate infusion.

The current studies also indicate that CPMM’s hypnotic actions in dogs are similar to that previously observed in rats. In both species, anesthetic induction is fast and hypnotic potency is high (hypnotic ED$_{50}$ approximately 0.8 and 0.69 mg/kg in dogs and rats, respectively).$^{23}$ The duration of hypnosis increases approximately linearly with the logarithm of the dose in both species with slopes for this relation that are similar (9.9 and 6.9 in dogs and rats, respectively).$^{25}$ In dogs and rats, hypnotic recovery times are independent of infusion duration and occur on similar time scales after 2-h continuous CPMM infusions (5.5 and 4.2 min in dogs and rats, respectively).$^{24}$

Cyclopropyl-methoxycarbonyl metomidate’s actions on the adrenocortical system are also similar in rats and dogs.$^{25}$ As in rats, adrenocortical responsiveness to ACTH$_{1-24}$ stimulation in dogs is suppressed during CPMM infusion. However after infusion, ACTH$_{1-24}$-stimulated plasma cortisol concentrations recover much more quickly after CPMM than after etomidate. The current studies in dogs further show that within 90 min after ending a 2-h infusion, adrenocortical responsiveness after CPMM is equivalent to that observed after propofol. These results suggest that with CPMM, any impact on adrenocortical steroid synthesis after single bolus administration or short-term continuous infusion is likely to be brief, clinically unimportant, and equivalent to the current clinical standard of care, propofol. These findings in dogs strongly support the further advancing CPMM to potential first in human phase 1 clinical trials.

The pharmacokinetic data presented in these studies are helpful for understanding the unique pharmacology of CPMM when compared with etomidate. To provide a visual comparison of the two drugs, figure 9 superimposes their normalized plasma concentrations as a function of time after either single bolus administration (fig. 9A) or single bolus followed by 2-h infusion (fig. 9B) and indicates the approximate normalized concentration ranges associated with medium anesthetic depth (i.e., anesthesia score of 3), full anesthetic recovery (i.e., anesthesia score of 0), and in vivo

**Fig. 7.** Adrenocortical response to adrenocorticotropic hormone (ACTH) stimulation after administration of cyclopropyl-methoxycarbonyl metomidate (CPMM) or propofol. ACTH$_{1-24}$-stimulated cortisol concentrations after administering a single bolus followed by 2-h infusion of CPMM or propofol. Infusions ended at time 0 min and ACTH$_{1-24}$ was administered at times 90 and 180 min. Plasma cortisol concentrations were measured in blood samples drawn 90 and 180 min after infusion termination. n = 4 dogs for each data point.
adrenolytic IC$_{50}$ value (i.e., adrenocortical half-inhibitory concentration).

After single bolus administration, the normalized plasma concentration of CPMM decreased much more quickly than that of etomidate (fig. 8A). Ten minutes after hypnotic bolus, CPMM’s normalized concentration was 33% of that of etomidate and (in contrast to etomidate) below that associated with full anesthetic recovery. This explains why dogs emerge more quickly after a CPMM bolus than after an etomidate bolus. One hour after the hypnotic bolus, CPMM’s normalized concentration was 7% of that of etomidate and (also in contrast to etomidate) well below its adrenolytic IC$_{50}$ value due to its far more rapid metabolism.

After single bolus followed by 2-h infusion, the normalized plasma concentration of CPMM initially decreased more quickly than that of etomidate and on the same time scale that we had observed after single bolus administration (fig. 8B). Thirty minutes after ending the hypnotic infusion, CPMM’s normalized concentration was 13% of etomidate’s normalized concentration and (in contrast to etomidate) below that associated with full anesthetic recovery. Beyond 30 min, the normalized concentration of CPMM remained lower than that of etomidate (and therefore reached the adrenolytic IC$_{50}$ value sooner), but the rate of decline was similar for the two drugs (half-life approximately 90 min) suggesting a common rate-controlling step. Previous studies with etomidate suggest that this slow step is the return of drug to the central compartment from a deep peripheral compartment into which drug had accumulated over time and was protected from metabolism.  

Similar to etomidate, CPMM produced myoclonus that is substantially attenuated (or eliminated) by midazolam. The incidence of myoclonus in our dogs was greater during CPMM infusion than during etomidate infusion (for all studies: 8 of 8 dogs vs. 2 of 4 dogs, respectively), and consequently, midazolam dosing was higher during the former (average midazolam dose in all studies: 0.4 ± 0.15 mg/kg vs. 0.13 ± 0.15 mg/kg, respectively). The higher midazolam dosing with CPMM as compared with etomidate (including in the behavioral recovery studies) indicates that the more rapid behavioral recovery seen with CPMM is not a manifestation of midazolam administration as the higher midazolam dosing is predicted to slow recovery. Rather, the significantly faster recovery after

Anesthesiology 2014; 121:1203-16

Campagna et al.
CPMM administration almost certainly reflects CPMM’s faster rate of metabolism.

Although we have not yet characterized the hypnotic potency of CPMM metabolite, the current studies suggest that it may be orders of magnitude lower than that of CPMM; complete hypnotic recovery occurs within minutes of terminating a 2-h CPMM infusion even as the metabolite persists at a concentration (in the blood) that is 10 times greater than that associated with CPMM-induced hypnosis. Such low hypnotic potency is also predicted by studies showing that CPMM metabolite’s potency for enhancing γ-aminobutyric acid receptor function is less than 1 of 1,000 that of CPMM (unpublished results, E. Pejo and D. E. Raines, Massachusetts General Hospital, March 2013).

The blood samples drawn for our pharmacokinetic measurements were taken from the venous circulation. For drugs that are metabolized rapidly in the periphery (i.e., extrahepatically), concentrations measured in venous blood may be lower than those measured in arterial blood because significant tissue metabolism occurs as the drugs transit from the venous to the arterial systems. For example, remifentanil concentrations in the venous circulation are approximately half that of the arterial circulation during infusion. Such arterial-venous concentration gradients can affect the values of pharmacokinetic and pharmacodynamic parameters derived from modeling, including increasing the calculated clearance and steady-state volume of distribution. In our data set, this gradient may also explain why the extrapolated initial concentration \( C_0 \) of CPMM in our bolus studies was only half that of etomidate even though the CPMM dose was higher. It may also explain why the same level of anesthetic depth was reached in our bolus studies with venous concentrations of CPMM that are only 1/2 to 1/3 those of etomidate.

Acknowledgments

The authors thank Scott Chappel, Ph.D., Annovation BioPharma, Cambridge, Massachusetts, for his helpful comments during the writing of this article, Robert J. Gutendorf, Ph.D., DMPK at Aclairo Pharmaceutical Development Group, Vienna, Virginia, for his assistance in modeling the pharmacokinetic data, and T. Scott Johnson, M.D., The Medicines Company, Parsippany, New Jersey, for critical reading of the article.

Supported by Annovation BioPharma, Inc., Cambridge, Massachusetts, and grant R01-GM087316 from the National Institutes of Health, Bethesda, Maryland, and the Department of Anesthesia, Critical Care, and Pain Medicine, Massachusetts General Hospital, Boston, Massachusetts.

Competing Interests

The Massachusetts General Hospital has published and submitted patent applications for cyclopropyl-methoxycarbonyl metomidate and related analogues. Dr. Raines is the lead inventor of this technology. He and his laboratory could receive compensation related to the development of this technology. Drs. Grayzel, Pojasek, Randle, and Raines have equity interests in Annovation BioPharma, Inc. Dr. Campagna is an employee of The Medicines Company (Parsippany, New Jersey), which has an equity interest in Annovation BioPharma, Inc.

Correspondence

Address correspondence to Dr. Raines: Department of Anesthesia, Critical Care, and Pain Medicine, Massachusetts General Hospital, 55 Fruit Street, GRB444, Boston, Massachusetts 02114. drainage@partners.org. Information on purchasing re-

Fig. 9. Normalized plasma hypnotic concentrations after (A) single bolus or (B) single bolus followed by 2-h hypnotic infusion. Plasma concentrations of each hypnotic were normalized to the concentration present during medium anesthesia depth (i.e., anesthesia score 3). For single-bolus-only studies, this was the plasma hypnotic concentration 30 s after bolus administration. For single bolus followed by 2-h hypnotic infusion studies, this was the plasma hypnotic concentration when the infusion ended. The approximate relative hypnotic concentrations associated with an anesthesia score of 3, full anesthetic recovery, and adrenolytic IC₅₀ (adrenocortical half-inhibitory concentration) are indicated by the shaded bars. CPMM = cyclopropyl-methoxycarbonyl metomidate.
prints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY’s articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

References
34. Liou M, Sonnen JM, Husain SS, Miller KW, Jurd R, Rudolph U, Eger EI II: R (+) etomidate and the photoactivatable R (+) azetidomide have comparable anesthetic activity in wild-type mice and comparably decreased activity in mice with a N265M point mutation in the γ-aminobutyric acid receptor β3 subunit. Anesth Analg 2005; 101:131–5


ANESTHESIOLOGY REFLECTIONS FROM THE WOOD LIBRARY-MUSEUM

Parke-Davis Medicated Throat Discs: Chloroforming That Throat “Tickle”

Formulated with capsicum, peppermint, anise, cubeb, licorice, linseed, acacia, and sugar, each Parke-Davis Medicated Throat Disc included 0.5 minim or roughly 0.03 ml of chloroform (lower right). This half-drop of chloroform provided some topical relief but also served as a solvent for the other “herbs and spices.” Targeting “singers, speakers, and smokers,” the box (upper left) for these throat discs promised relief from “coughs due to colds, hoarseness, irritation of the throat, etc.” Each Disc contains not more than 1/2 minim of CHLOROFORM.

Each Disc contains not more than 1/2 minim of CHLOROFORM. Free from injurious drugs.

George S. Bause, M.D., M.P.H., Honorary Curator, ASA’s Wood Library-Museum of Anesthesiology, Schaumburg, Illinois, and Clinical Associate Professor, Case Western Reserve University, Cleveland, Ohio. UJYC@aol.com.