Cyclopropyl-methoxycarbonyl Metomidate

Studies in a Lipopolysaccharide Inflammatory Model of Sepsis

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ABSTRACT

Background: Cyclopropyl-methoxycarbonyl metomidate (CPMM) is a rapidly metabolized etomidate analog that is currently in clinical trials. The goal of this study is to assess CPMM's potential value as an anesthetic agent for use in patients with sepsis by defining its actions in an acute inflammatory model of sepsis.

Methods: *Escherichia coli* lipopolysaccharide (1 mg/kg) was injected intravenously into Sprague–Dawley rats. Thirty minutes later, CPMM, etomidate, or vehicle (n = 8 per group) was infused for 1 h. Plasma adrenocorticotropic hormone, corticosterone, and cytokine (interleukin-1β, interleukin-6, interleukin-10, and tumor necrosis factor-α) concentrations were measured before, during, and after infusion.

Results: After lipopolysaccharide injection, adrenocorticotropic hormone concentrations changed similarly over time in all three groups. Compared with vehicle group rats, CPMM group rats had significantly lower corticosterone concentrations at only a single study time point during infusion and no significant differences in cytokine concentrations at any time during the study period. Compared with etomidate group rats, CPMM group rats had significantly higher corticosterone concentrations (up to nine-fold) during and after hypnotic infusion. Cytokine concentrations in CPMM group rats and vehicle group rats were not significantly different, but they were significantly lower than those in etomidate group rats. Postinfusion mortality was 40% in etomidate group rats and 0% in CPMM and vehicle group rats.

Conclusion: Compared with etomidate, CPMM produces less adrenocortical suppression, lower plasma cytokine concentrations, and improved survival in a lipopolysaccharide inflammatory model of sepsis. These results suggest that CPMM may be a safer alternative to etomidate in patients with sepsis. **(Anesthesiology 2015; 123:368-76)**

SEPSIS, defined as the systemic inflammatory response to infection, is a life-threatening disease that is often associated with acute organ dysfunction and the need for intensive care treatment.[1] In the United States alone, more than 750,000 cases are recorded each year accounting for 10% of all intensive care unit admissions.[1,2] Mortality is high with one out of three patients dying from complications.[2–4] The incidence of sepsis has been steadily increasing over the last decade, as have the total costs associated with treatment, which now exceed $20 billion annually.[2,5] Patients with sepsis frequently require general anesthesia for important therapeutic interventions such as intubation to assist breathing or surgery to treat the underlying infectious source. This task can be difficult to achieve safely as patients with sepsis are often hemodynamically unstable and therefore particularly vulnerable to the cardiovascular depression produced by most anesthetic agents.

Etomidate is an imidazole-based anesthetic agent that has long been favored for its minimal effects on cardiovascular function.[6–8] However, its use as a prolonged continuous infusion agent to maintain anesthesia—particularly in patients with sepsis—has long been abandoned because it potently suppresses the synthesis of adrenocortical steroids that serve to restore homeostasis and improve survival.[9–12] More recently, the administration of even a single bolus

What We Already Know about This Topic

- Patients with sepsis are often hemodynamically unstable and vulnerable to cardiovascular depression produced by anesthetic agents
- Etomidate minimally affects cardiovascular function but potently suppresses synthesis of adrenocortical steroids that restore homeostasis and improve survival
- Cyclopropyl-methoxycarbonyl metomidate is an etomidate analog that undergoes hydrolysis by nonspecific esterases, as a result of which it produces brief hypnosis that is independent of infusion duration and adrenocortical function recovers rapidly

What This Article Tells Us That Is New

- A 1-h cyclopropyl-methoxycarbonyl metomidate infusion produced less suppression of adrenocortical steroid synthesis, less elevated plasma inflammatory cytokine concentrations, and lower mortality than did an etomidate infusion in an *Escherichia coli* lipopolysaccharide rat model of sepsis

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dose for anesthetic induction has become highly controversial because it can suppress adrenocortical function for more than a day and may increase mortality in the critically ill.[13–15] With the goal of reducing etomidate's impact on adrenocortical function while preserving its favorable hemodynamic profile, we began a program to develop "soft" (*i.e.*, rapidly metabolized) analogs of etomidate.[16–22] Of the more than a dozen analogs produced and studied in our laboratory, cyclopropyl-methoxycarbonyl metomidate (CPMM) exhibited the most favorable pharmacology in animals.[19–21] This drug (which is also known as ABP700) has now reached clinical trials in healthy human volunteers.

The purpose of the present studies was to assess CPMM's potential value as a hypnotic agent for use in patients with sepsis by defining its actions in an experimental animal model of acute inflammation. Our hypothesis was that compared with etomidate, CPMM would produce less suppression of adrenocortical steroid synthesis and therefore lower elevations of deleterious proinflammatory cytokines in this experimental model. Such findings would suggest that CPMM might be safer than etomidate as a hypnotic for patients with sepsis (or other inflammatory conditions) and provide a basis for its clinical testing in this patient population. To test this hypothesis, we determined the impact of 1-h infusions of etomidate and CPMM on the pituitary, adrenocortical, and cytokine responses and on the arterial blood chemistries of rats challenged with *Escherichia coli* lipopolysaccharide.

Materials and Methods

Animals

All studies were conducted with the approval of and in accordance with the regulations of the Institutional Animal Care and Use Committee at Massachusetts General Hospital (Boston, Massachusetts). Adult male Sprague–Dawley rats (330 to 430 g) were purchased from Charles River Laboratories (USA) with femoral arterial catheters implanted by the vendor. Rats were individually caged in the animal care facility of the Massachusetts General Hospital Center for Comparative Medicine with controlled temperature (21 ± 2°C) and light–dark cycles (7 AM to 7 PM). Food and water were provided *ad libitum*. The sample size used in each group (six to eight rats) was determined based on our previous experience and is in the range used by other laboratories for analogous studies.[23–26]

Drugs and Chemicals

Cyclopropyl-methoxycarbonyl metomidate was synthesized by Aberjona Laboratories (USA) as previously described.[21] Etomidate was obtained from Bachem Americas (USA). Both drugs were solubilized in phosphate-buffered saline purchased from ATCC (SCRR-2201, USA) with the pH adjusted to 2.5. Lipopolysaccharide from *E. coli* serotype 055:B5 was purchased from Sigma-Aldrich (USA). Heparin was obtained from Hospira (USA).

Hypnotic Drug Infusion Protocol

Animals were randomized into etomidate, CPMM, or vehicle groups (n = 8 rats per group). Throughout the experiment, rats were restrained in a 9-inch long, 3-inch diameter acrylic tube with ports for oxygen supplementation (100%, 2 l/min), arterial line access, and tail exit. To reduce the influence of diurnal variations in corticosterone concentrations, all experiments were begun at 8:30 AM. After weighing and placement of a 24-gauge tail vein catheter for drug administration (performed with brief isoflurane anesthesia), rats were allowed to recover and acclimate to restriction for 2 h. Lipopolysaccharide (1 mg/kg) was then injected intravenously. Thirty minutes later, a 1-h intravenous infusion of etomidate, CPMM, or vehicle was started. To achieve and maintain equivalent hypnotic depths among the etomidate and CPMM groups, previously established continuous infusion protocols were used.[20] These protocols were previously shown to maintain the electroencephalographic burst suppression ratio at 80% in the presence of 1% isoflurane and produce a steady-state anesthetic depth that is 1.3 times that required to produce immobility to a standard noxious stimulus in the absence of isoflurane. The total doses of CPMM and etomidate delivered during 1-h infusions were 71 and 19 mg/kg, respectively. Etomidate and CPMM infusions were prepared at concentrations of 2.0 and 7.6 mg/ml, respectively, to administer equal volumes (*i.e.*, 9.4 ml/kg) of vehicle during the 1-h infusion period. Vehicle-only rat groups received 9.4 ml/kg of the vehicle during the 1-h infusion period. Animals were allowed to breathe spontaneously throughout the study.

Sample Analysis

Arterial blood samples (0.4 ml) were collected from the femoral arterial catheter immediately before and 15, 30, 60, 90, 120, 180, and 240 min after lipopolysaccharide injection. This volume was replaced with normal saline (1 ml). Samples were immediately mixed with EDTA (3 mg per sample) and then centrifuged at 1,530*g* at 4°C for 15 min. The resultant plasma fraction was separated and stored at −80°C until analyzed. Corticosterone and adrenocorticotropic hormone (ACTH) concentrations in each sample were analyzed using enzyme-linked immunosorbent assay kits purchased from Immunodiagnostics Systems (USA) and MD Bioproducts (USA), respectively. Plasma concentrations of tumor necrosis factor-α, interleukin (IL)-1β, IL-6, and IL-10 were measured using a MILLIPLEX® MAP Rat Cytokine/Chemokine Magnetic Bead Panel from EMD Millipore (USA).

Additional arterial blood samples (0.2 ml) were drawn into heparinized syringes before and 30, 90, 120, 180, and 240 min after lipopolysaccharide administration for arterial blood chemistry analysis. This volume was replaced with normal saline. Analysis of samples was performed within 5 min of sample collection using a VetScan i-STAT 1 handheld analyzer from Abaxis (USA) with CG4+ Cartridges from Abbott Laboratories (USA).

Determination of Median Hypnotic Doses (ED_{50}s)

To confirm equal and adequate anesthetic depths in both drug groups, hypnotic potencies for continuous infusions were determined using a loss of righting reflex (LORR) assay (n = 5 rats/hypnotic). The initial infusion rate for each hypnotic was 40 to 50% of the previously established values required to produce immobility.[20] After 40 min of infusion, rats were turned supine and their abilities to right (*i.e.*, turn themselves spontaneously back onto all four paws) was assessed. A rat was judged to have LORR if it failed to right for at least 1 min. If a rat was considered not to have righting reflex, the infusion rate was increased by 20%. After 40-min equilibration time, rats were again assessed for LORR. Infusion rates were increased every 40 min until a rat showed LORR for at least 1 min. The hypnotic ED_{50} was defined as the average of the highest infusion rate that failed to produce LORR and the subsequent higher rate that produced LORR.

Statistical Analysis

All data are reported as mean ± SD. Statistical analysis was performed using GraphPad Prism 5.0 (USA). Our goal in these studies was to compare the effects of different drug treatments (*i.e.*, etomidate, CPMM, or vehicle) at each time point after lipopolysaccharide administration. Therefore, we used a repeated measures two-way ANOVA. *Post hoc* analyses were conducted where statistically significant main effects or interaction effects were observed, with Bonferroni adjustment for multiple comparisons. The two factors were group and time after lipopolysaccharide administration. All statistical analyses were two tailed with *P* value less than 0.05 considered statistically significant.

Results

The Impact of 1-h Infusions of CPMM and Etomidate on Plasma Concentrations of ACTH and Corticosterone in Lipopolysaccharide-treated Rats

Baseline plasma ACTH and corticosterone concentrations before lipopolysaccharide injection or hypnotic infusion (time = 0 min) were not significantly different among the three rat groups (CPMM, etomidate, and vehicle) with mean values of 31 ± 41 pg/ml and 95 ± 96 ng/ml, respectively (fig. 1). During the first 30 min after injecting lipopolysaccharide and before starting hypnotic infusions, plasma concentrations of ACTH and corticosterone increased similarly in all three groups reaching mean values of 208 ± 113 pg/ml and 455 ± 183 ng/ml, respectively, 30 min after lipopolysaccharide injection (time = 30 min). However, after starting the 1-h infusions of CPMM, etomidate, or vehicle, plasma corticosterone (but not ACTH) concentrations among the three rat groups diverged.

In vehicle group rats, plasma corticosterone concentrations continued to increase into the 1-h infusion period. Its concentration reached an approximate steady-state value with a mean that ranged from 698 ± 291 to 861 ± 315 ng/ml between 60 and 240 min after injecting lipopolysaccharide.

In CPMM group rats, plasma corticosterone concentrations also continued to increase into the infusion period before reaching a similar steady-state value. However, the rate of increase was less than that seen in vehicle group rats as the plasma corticosterone concentration midway through the 1-h infusion (time = 60 min) in CPMM group rats was only half that of vehicle group rats (449 ± 227 *vs.* 836 ± 271 ng/ml, respectively; $P < 0.05$). However, at all other time points during the study, there were no significant differences in plasma corticosterone concentrations between CPMM and vehicle rat groups.

In etomidate group rats, plasma corticosterone concentrations progressively decreased during the infusion period. By the end of the 1-h infusion (time = 90 min), plasma corticosterone concentrations in etomidate group rats were only 8.7 ± 5.2% and 10 ± 6.4% of those present in vehicle and CPMM group rats, respectively. After the infusion was complete, plasma corticosterone concentrations in etomidate group rats remained significantly depressed relative to both CPMM and vehicle groups for another 90 to 150 min.

The Impact of 1-h Infusions of CPMM and Etomidate on Plasma Concentrations of Cytokines in Lipopolysaccharide-treated Rats

Baseline plasma cytokine concentrations before lipopolysaccharide injection were not significantly different among the three rat groups with mean values of 134 ± 72 pg/ml for IL-1β, 153 ± 114 pg/ml for IL-6, 202 ± 39 pg/ml for IL-10, and 8.9 ± 2.2 pg/ml for tumor necrosis factor-α (fig. 2). After lipopolysaccharide injection, plasma cytokine concentrations increased by up to three orders of magnitude in the three rat groups. There were no significant differences in plasma cytokine concentrations between CPMM and vehicle group rats at any time point during the study. However, plasma IL-1β, IL-6, and IL-10 concentrations in etomidate group rats were significantly higher at various postinfusion time points than those in either CPMM or vehicle group rats. For IL-1β and IL-6, plasma concentrations remained higher to the end of our study (time = 240 min).

The Impact of 1-h Infusions of CPMM and Etomidate on Arterial Blood Chemistry Values in Rats

Arterial blood chemistries were also measured intermittently in all three rat groups. In addition, we measured arterial blood chemistries in a control group (n = 6) that received a vehicle infusion but was not injected with lipopolysaccharide (vehicle-no lipopolysaccharide group) to establish normal values for comparison. In all four rat groups (CPMM, etomidate, vehicle, and vehicle-no lipopolysaccharide), baseline chemistry values at the time of lipopolysaccharide injection (time = 0 min) were not significantly different with mean values of 7.46 ± 0.02 (pH), 290 ± 12 mmHg (pO_2), 44 ± 3 mmHg (pCO_2), 6.8 ± 0.5 mM (base excess), 30.7 ± 0.8 mM (bicarbonate), and 0.72 ± 0.15 mM (lactate).

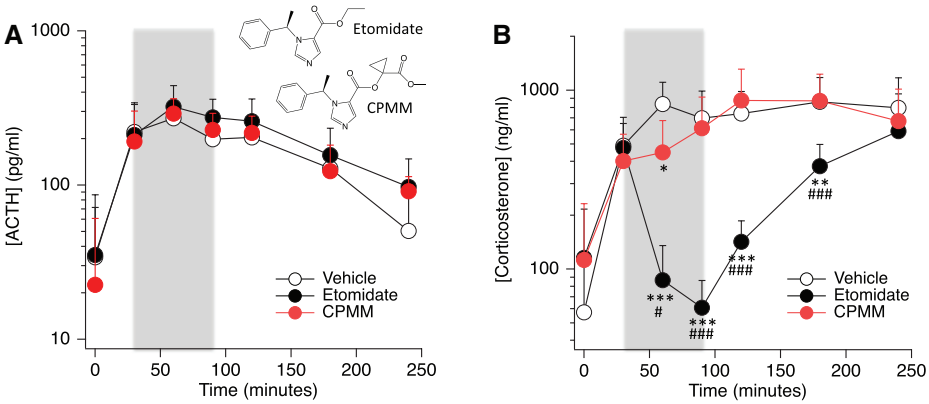

Fig. 1. Impact of 1-h infusions of cyclopropyl-methoxycarbonyl metomidate (CPMM), etomidate, or vehicle on plasma (*A*) adrenocorticotropic hormone (ACTH) concentrations and (*B*) corticosterone concentrations. Lipopolysaccharide was administered at time = 0 min. Infusions ran from time = 30 min until time = 90 min (*shaded period*). Each *data point* is the mean (± SD) value from eight rats. The *inset* in *A* shows the chemical structures of etomidate and CPMM. Statistically significant interactions between group and time were observed for corticosterone concentrations but not for ACTH concentrations. **P* < 0.05 etomidate *versus* vehicle; ***P* < 0.01 etomidate *versus* vehicle; ****P* < 0.001 etomidate *versus* vehicle; #*P* < 0.05 etomidate *versus* CPMM; ###*P* < 0.001 etomidate *versus* CPMM.

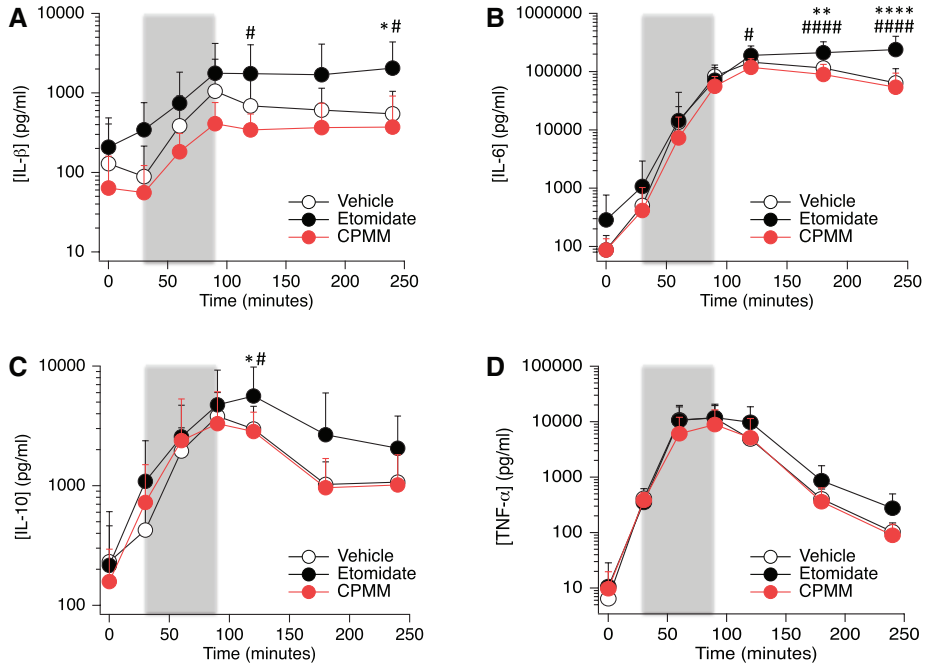

Fig. 2. Impact of 1-h infusions of cyclopropyl-methoxycarbonyl metomidate (CPMM), etomidate, or vehicle on plasma (*A*) interleukin (IL)-1β concentrations, (*B*) IL-6 concentrations, (*C*) IL-10 concentrations, and (*D*) tumor necrosis factor-α (TNF-α) concentrations. Lipopolysaccharide was administered at time = 0 min. Infusions ran from time = 30 min until time = 90 min (*shaded period*). Each *data point* is the mean (± SD) value from eight rats. Statistically significant interactions between group and time were observed for IL-6 concentrations but not for the other cytokine concentrations. **P* < 0.05 etomidate *versus* vehicle; ***P* < 0.01 etomidate *versus* vehicle; *****P* < 0.0001 etomidate *versus* vehicle; #*P* < 0.05 etomidate *versus* CPMM; ####*P* < 0.0001 etomidate *versus* CPMM.

After lipopolysaccharide injection and during infusion, a metabolic acidosis developed in the CPMM, etomidate, and vehicle group rats as reflected by significantly lower blood pHs (along with lower base excesses and bicarbonate concentrations) and significantly higher lactate concentrations in these three groups as compared with the vehicle-no lipopolysaccharide rat group (fig. 3). Blood pCO_2 was also significantly higher during the 1-h infusion period in CPMM and etomidate group rats as compared with that measured in the two vehicle groups, indicating an additional respiratory component to the acidosis. During the 1-h infusions, lactate concentrations increased similarly in both CPMM and etomidate group rats but less than those in vehicle group rats. After the infusions ended, lactate concentrations

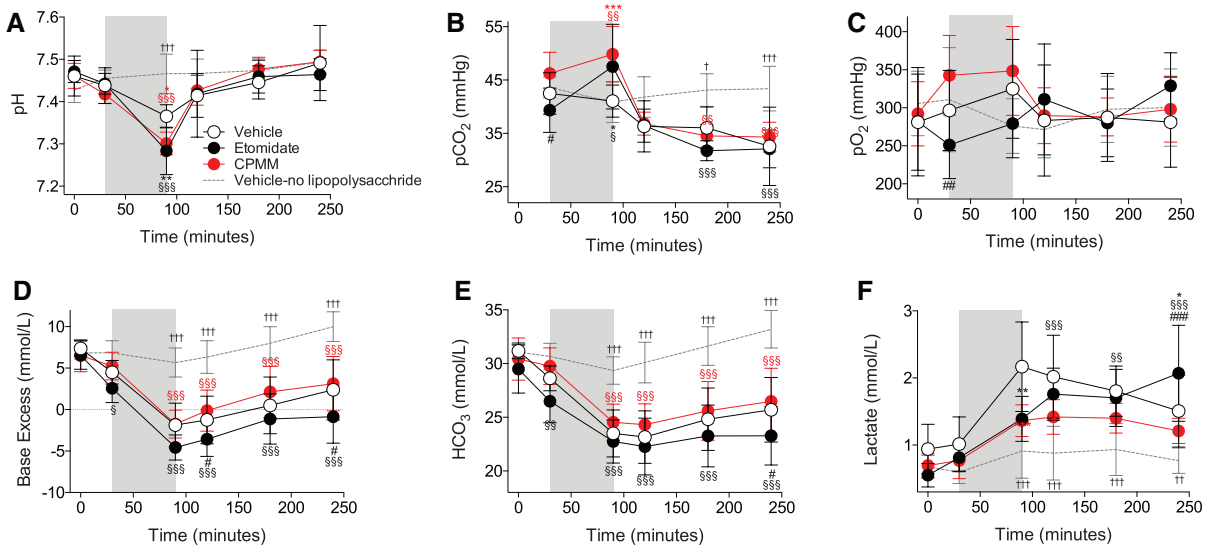

Fig. 3. Impact of 1-h infusions of cyclopropyl-methoxycarbonyl metomidate (CPMM), etomidate, vehicle, or vehicle-no lipopolysaccharide on arterial blood (*A*) pH, (*B*) pCO_2, (*C*) pO_2, (*D*) base excess, (*E*) bicarbonate, and (*F*) lactate concentrations. Lipopolysaccharide was administered at time = 0 min to CPMM, etomidate, and vehicle group rats. Normal saline was administered at time = 0 min to the vehicle-no lipopolysaccharide group rats. Infusions ran from time = 30 min until time = 90 min (*shaded period*). Each *data point* is the mean (± SD) value from six rats (vehicle-no lipopolysaccharide group) or eight rats (all other groups). Statistically significant interactions between group and time were observed for all values. **P* < 0.05 etomidate *versus* vehicle; ***P* < 0.01 etomidate *versus* vehicle; **P* < 0.05 CPMM *versus* vehicle; ****P* < 0.001 CPMM *versus* vehicle; #*P* < 0.05 CPMM *versus* etomidate; ##*P* < 0.01 CPMM *versus* etomidate; ###*P* < 0.001 CPMM *versus* etomidate; §*P* < 0.05 etomidate *versus* vehicle-no lipopolysaccharide; §§*P* < 0.01 etomidate *versus* vehicle-no lipopolysaccharide; §§§*P* < 0.001 etomidate *versus* vehicle-no lipopolysaccharide; §§*P* < 0.01 CPMM *versus* vehicle-no lipopolysaccharide; §§§*P* < 0.001 CPMM *versus* vehicle-no lipopolysaccharide; †*P* < 0.05 vehicle *versus* vehicle-no lipopolysaccharide; ††*P* < 0.01 vehicle *versus* vehicle-no lipopolysaccharide; †††*P* < 0.001 vehicle *versus* vehicle-no lipopolysaccharide.

trended higher in etomidate (but not CPMM) group rats reaching a value 2.1 ± 0.7 mM. This was significantly higher than those of any other group.

Mortality after 1-h Infusions of Vehicle, CPMM, and Etomidate

All 24 rats in the three groups that received lipopolysaccharide (*i.e.*, CPMM, etomidate, and vehicle) and the six rats in the vehicle-no lipopolysaccharide group survived the 4-h study period. However, three of the eight rats in the etomidate group unexpectedly died overnight, whereas none of the rats in the other three groups died. A *post hoc* analysis of the etomidate group data revealed no difference at any time point in plasma ACTH concentrations between the three rats that died and the five that survived and recovered from the lipopolysaccharide challenge (fig. 4A). However, significant differences were found in corticosterone (fig. 4B) and cytokine (fig. 4, C–F) concentrations during the etomidate infusion or postinfusion periods. In particular, rats in the etomidate group that died had plasma concentrations of corticosterone, IL-1β, and IL-6 that were significantly higher than those that survived at the end of our study (time = 240 min). In addition, etomidate group rats that died had significantly lower blood pH and higher lactate concentrations at the end of our study than those that survived (fig. 4, G and H).

Hypnotic ED_{50}s of CPMM and Etomidate

Continuous infusion of both CPMM and etomidate produced hypnosis at infusion rates that were lower than those required to produce immobility.[20] The mean (± SD) ED_{50}s for hypnosis were 0.56 ± 0.09 mg kg^{-1}·min^{-1} for CPMM and 0.15 ± 0.03 mg kg^{-1}·min^{-1} for etomidate (fig. 5). The steady-state infusion rates used in this study were 2.0 (CPMM) and 1.7 (etomidate) times higher than the hypnotic ED_{50}s and sufficient to produce hypnosis. In addition, these findings confirm that the infusion protocol used was suitable in maintaining approximately equivalent and "clinically" relevant hypnotic depths in both drug groups.

Discussion

These are the first studies to define the pharmacology of the soft etomidate analog CPMM in an animal model of disease. With 1-h infusions, we found that CPMM produced less suppression of adrenocortical steroid synthesis, reduced elevations of the cytokines IL-1β, IL-6, and IL-10, and lower mortality after lipopolysaccharide challenge than etomidate.

The use of prolonged etomidate infusions has been abandoned by clinicians, and the administration of even a single bolus dose of etomidate has become highly controversial because of concerns that they may increase morbidity and mortality, particularly in the setting of sepsis.[9,11,13,14,27–32] The underlying cause for etomidate's deleterious effects

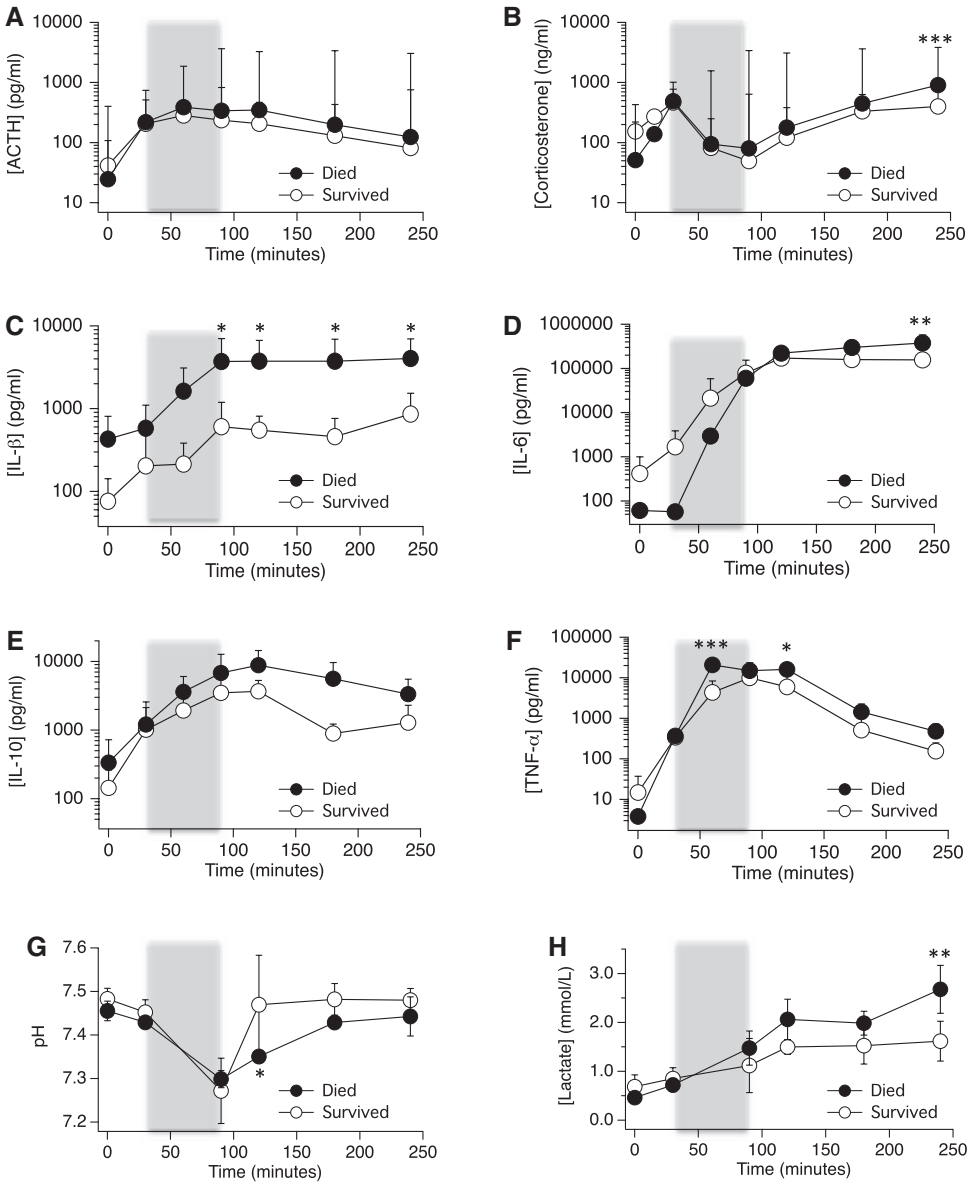

Fig. 4. Comparison of etomidate rats that died *versus* survived: effects on plasma (*A*) adrenocorticotropic hormone (ACTH) concentrations, (*B*) corticosterone concentrations, (*C*) interleukin (IL)-1β concentrations, (*D*) IL-6 concentrations, (*E*) IL-10 concentrations, (*F*) tumor necrosis factor-α (TNF-α) concentrations, blood pH (*G*), and blood lactate concentrations (*H*). Lipopolysaccharide was administered at time = 0 min. Infusions ran from time = 30 min until time = 90 min (*shaded period*). Each *data point* is the mean (± SD) value from three rats (died) or five rats (survived). No statistically significant interactions between group and time were observed. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

is widely believed to be its ability to inhibit the activity of 11β-hydroxylase and thus suppress the biosynthesis of adrenocortical steroids even when ACTH levels are high. These steroids are critical components of the counter-regulatory mechanism that serves to reduce the production of proinflammatory cytokines that contribute to the pathophysiology of sepsis.[33,34] Because etomidate is approximately two orders of magnitude more potent an inhibitor of adrenocortical steroid synthesis than it is a hypnotic, a hypnotic dose of etomidate represents a massive overdose with respect to that needed to suppress the adrenocortical steroid production.[35] Consequently, after administering a hypnotic etomidate dose, multiple elimination half-lives must pass before the plasma etomidate concentration decreases below that which inhibits adrenocortical function. It is within this mechanistic framework that our laboratory developed CPMM.[21] CPMM is an analog of etomidate that, similar to remifentanil and esmolol, was specifically designed to be susceptible to hydrolysis by nonspecific esterases and to form an essentially inactive carboxylic acid metabolite. Because of this structural design feature, CPMM produces hypnosis that is brief and independent of infusion duration, and adrenocortical function recovers rapidly even after prolonged continuous CPMM infusion.[18,20]

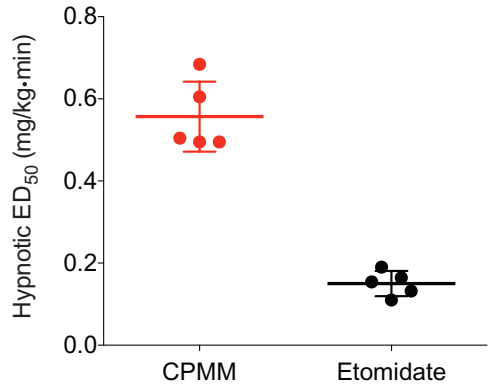

Fig. 5. Median hypnotic doses for continuous infusions of cyclopropyl-methoxycarbonyl metomidate (CPMM) and etomidate. Each *data point* represents the median hypnotic dose (*i.e.*, the median dose to induce loss of righting reflex) obtained in a single rat. The *bars* indicate the mean (± SD) values.

The present studies show that in a lipopolysaccharide inflammatory model of sepsis, 1-h infusions of CPMM and etomidate differentially modulate adrenocortical steroid synthesis. During infusions of etomidate, we found that plasma corticosterone concentrations progressively decreased. At the end of the 1-h etomidate infusion, the corticosterone concentration was 1/8th of that present at the start of the infusion. As the half-life of corticosterone in the rat is approximately 20 min, these results indicate that corticosterone synthesis was essentially completely inhibited during the etomidate infusion.[36] Plasma corticosterone concentrations in etomidate group rats then recovered toward those of vehicle group rats during the next approximately 2 h, presumably reflecting hepatic elimination of etomidate. In contrast, plasma corticosterone concentrations progressively increased in CPMM group rats although at a slower rate than those in vehicle group rats. By the end of the 1-h infusion, plasma corticosterone concentrations in CPMM and vehicle group rats were not significantly different. This suggests that although CPMM also suppresses lipopolysaccharide-induced corticosterone synthesis, the magnitude is significantly less than that produced by etomidate.

In addition to differentially affecting adrenocortical steroid synthesis, 1-h infusions of CPMM and etomidate also differentially affected cytokine production. CPMM had no significant effects on the plasma concentrations of any of the four measured cytokines after lipopolysaccharide injection, whereas etomidate significantly increased concentrations of IL-1β, IL-6, and IL-10. For the proinflammatory cytokines IL-1β and IL-6, these increases remained unabated to the end of our 4-h study. As corticosteroids serve a counter-regulatory role to suppress the production of cytokines in the setting of inflammation, we speculate that the higher plasma cytokine concentrations measured in etomidate group rats resulted from the profound and prolonged inhibition of corticosterone production that occurred with 1-h etomidate infusions. Analogous "pharmacological adrenalectomy" by mifepristone or surgical adrenalectomy similarly increases cytokine levels after lipopolysaccharide administration.[37–39]

Blood chemistry analysis revealed similarities during infusions and differences after infusions between CPMM and etomidate group rats. During 1-h infusions, rats in both groups exhibited similarly increased pCO_2 and reduced lactate concentrations, consistent with comparable levels of hypnotic-induced depression of ventilation and metabolic activity, respectively. However, at the end of our study, etomidate group rats had higher lactate concentrations along with lower bicarbonate concentrations and base excesses than CPMM group rats.

Mortality was not one of our intended study endpoints; nevertheless, we observed that 40% of the rats (three out of eight rats) in the etomidate group died overnight despite emerging from anesthesia and surviving the study period. This contrasts with the 0% mortality among rats in all other groups. A *post hoc* subgroup analysis revealed that compared with etomidate group rats that survived, etomidate group rats that died had a more profound and persistent metabolic acidosis and inflammatory response as reflected by their significantly lower blood pHs, higher blood lactate levels, and greater plasma concentrations of the proinflammatory cytokines IL-1β and IL-6 at the end of the 4-h study period.

Although the modest and brief reduction in plasma corticosterone concentrations by CPMM was not associated with increased cytokine concentrations, persistent changes in blood chemistry, or increased mortality, it should be noted that we infused this hypnotic for only 1 h. We do not know whether longer CPMM infusions might have produced more profound and/or prolonged suppression of adrenocortical steroid synthesis with resultant increased mortality. We also do not know whether supportive care (*e.g.*, the administration of vasopressors or steroids) might have reduced the mortality in etomidate group rats. Finally, our inflammatory model lacks the infectious focus and commonly occurring comorbidities of clinical sepsis. Thus, we do not know whether other animal models of sepsis or studies in man might lead to different conclusions. Additional studies are necessary to answer these important questions.

In summary, a 1-h CPMM infusion produces less adrenocortical suppression than a 1-h etomidate infusion and (unlike etomidate) without increasing either plasma cytokine concentrations or mortality in a lipopolysaccharide rat model of sepsis. These results suggest that CPMM may be a safer alternative to etomidate for the induction or short-term maintenance of anesthesia in the setting of sepsis.

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Competing Interests

The Massachusetts General Hospital has published and submitted patent applications for cyclopropyl-methoxycarbonyl metomidate and related analogs. Dr. Raines is the lead inventor of this technology. He and his laboratory could receive income related to the development of this technology. This technology has been licensed to Annovation BioPharma, Inc. (Cambridge, Massachusetts). Dr. Raines has an equity interest in Annovation BioPharma, Inc. All other authors have no conflicts of interest.

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ANESTHESIOLOGY REFLECTIONS FROM THE WOOD LIBRARY-MUSEUM

Monkshoods: Heart-stopping Neurotoxins behind Hanaoka's *Mafutsusan* (Year 1804, Part 2)

Widely regarded as the source for the deadliest Himalayan arrow poison, "monkshoods" (*left*) have flowers which resemble a monk's hood or cowl (*right*). The deadly Blue Aconite is a monkshood with blue flowers, the color of which was likely reflected in the partially swallowed poison lodged eternally in "blue-throated Shiva," Hinduism's "God of All Poisons." For mortals, just touching aconite to the gums might simultaneously alleviate a toothache and benumb the fingers and mouth. Beyond their neurotoxicity, such species of the *Aconitum* genus are cardiotoxic and can slow the heart rate to a standstill—a useful attribute when poisoning wolves ("Wolfsbane" is another aconitic nickname). To minimize patient mortality, Ayurvedic physicians typically soaked such *Aconitum* species in the urine or milk of sacred cows in order to weaken the poison. For pioneers of anesthesia such as China's Hua Tuo (c. 140–c. 208) and Japan's Seishū Hanaoka (1760–1835), the numbing aconitine of monkshoods provided the "pain dulling" portion of a primitive balanced anesthetic. (Copyright © the American Society of Anesthesiologists, Inc.)

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