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Metabolite-inactive etomidate analogues alleviating suppression on adrenal function in Beagle dogs

Jun Yang [a,1], Yi Kang [a,1], Bin Wang [a], Linghui Yang [a], Jin Liu [a,b], Wensheng Zhang [a,b,*]

[a] Laboratory of Anesthesia and Critical Care Medicine, Translational Neuroscience Centre, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, PR China
[b] Department of Anesthesiology, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, PR China

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ABSTRACT

Owing to rapid generation in body, the metabolites of etomidate softdrug are able to accumulate in either the brain or periphery and subsequently affect the recovery from anaesthesia or cause corticosteroid suppression. This study was designed to investigate the ability of two etomidate analogues (ET-26, ET-42) with inactive metabolites to provide anaesthesia with lesser corticosteroid suppression. The 50% effective dose (ED_{50}) of ET-26, ET-42, Etomidate, MOC-ET (an etomidate softdrug) and CPMM (an improved etomidate softdrug) required to induce anaesthesia intravenously in Beagle dogs were 1.44 mg/kg, 0.72 mg/kg, 0.43 mg/kg 23.12 mg/kg and 0.59 mg/kg, respectively. After adrenocorticotropic hormone (ACTH) stimulation, the serum concentrations of cortisol and corticosterone in the ET-26, ET-42 and CPMM groups were similar to those of controls, and significantly higher than those of the etomidate and MOC-etomidate groups ($P < 0.05$). Furthermore, no significant differences in serum concentrations of cortisol and corticosterone after ACTH-stimulation between ET-26, ET-42, CPMM, and blank control groups were observed. In this study, anaesthetic potencies of ET-26 (ED_{50} = 1.44 mg/kg) and ET-42 (ED_{50} = 0.72 mg/kg) were determined. Both analogues can significantly reduce the corticosteroid suppression *in vivo*. Metabolite-inactive etomidate derivatives with slow metabolism might provide a novel strategy to improve Etomidate associated corticosteroid suppression.

1. Introduction

Etomidate is one of the most widely used intravenous anaesthetics. Etomidate has several key pharmacological properties, including rapid onset of action and recovery from anaesthetic effects, a wide therapeutic index and a short context-sensitive half-life, in addition to providing cardiovascular stability (Nauta et al., 1983; Giese et al., 1985; Tomlin et al., 1998), making this agent an ideal intravenous general anaesthetic. However, etomidate has also been reported to cause adrenal toxicities in patients who are critically ill (Wagner and White, 1984; Lundy et al., 2007), owing to inhibition of corticosteroid synthesis (Duthie et al., 1985). Data from several studies have demonstrated this risk of adrenal insufficiency. Whether or not etomidate should be used in clinical practice, especially in patients who are critically ill, is therefore a matter of some debate (Cotton et al., 2008). The generally good safety profile and cardiovascular stability, however, do make etomidate a useful agent for certain patients, such as those undergoing surgery owing to heart failure and elderly patients (Bovill, 2006). Thus, an unmet need exists to reduce corticosteroid suppression of Etomidate and therefore expand the potential clinical applications of etomidate.

In the past 7 years, etomidate soft drugs with reduced corticosteroid suppression, such as MOC-ET (Cotten et al., 2009) and CPMM (Husain et al., 2012), have been developed. Whether the softdrug design enables this goal to be achieved will depend not only on the rate of metabolism of prototype molecule, but also on the activity of metabolite. Etomidate metabolism in humans depends on hepatic esterase activity, which hydrolyzes the drug to etomidate acid and an ethanol-leaving group. Etomidate acid is excreted mostly in urine and, to a lesser degree, in bile (Heykants et al., 1975). Etomidate acid does not exhibit any obvious activity, while the metabolite of MOC-ET (MOC-ECA) shows corticosteroid inhibition and central inhibition. MOC-ET can be hydrolyzed rapidly by wide spread hydrolytic enzymes, which are abundant in the brain, to generate MOC-ECA, which is largely unable to pass through the blood-brain barrier owing to its increased polarity. As a result, MOC-ECA accumulates in brain. MOC-ECA has been demonstrated to have a hypnotic potency that is 350-fold less than that of MOC-ET, resulting in a significantly increase burst suppression ratio (BSR) after termination of the infusion in rats (Pejo et al., 2012a). In addition, MOC-ECA can also produce corticosteroid suppression to a certain extent (about 1/300 of the potency of MOC-etomidate) (Ge et al., 2012a) and probably

* Corresponding author at: Laboratory of Anesthesia and Critical Care Medicine, Translational Neuroscience Centre, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, PR China.
E-mail address: zhang_ws@scu.edu.cn (W. Zhang).
[1] Contributed equally to this work.

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produces conspicuous corticosteroid suppression owing to its rapid generation and accumulation in the periphery. The chemical structure of metabolite of CPMM, CPMM-ECA, is similar to that of MOC-ECA. (Fig.1 a.). Experiments have demonstrated that BSR does not increase significantly after termination of CPMM-ECA infusion in rats (Ge et al., 2012b), although whether or not CPMM-ECA suppresses serum corticosteroid levels remains unknown.

In this study, we used strategy distinct from softdrug design to improve the profile of etomidate. We hypothesized that if the corticosteroid suppression of etomidate analogues and its metabolites can be prevented, even if these analogues have slow metabolic characteristic, it could not exhibit significant corticosteroid suppression. Based on this hypothesis, two etomidate analogues ET-26 and ET-42 were studied in this study.

2. Materials and Methods

2.1. Animal and Drugs

All experiments were approved by the Scientific Research Committee and the Institutional Animal Experimental Ethics Committee, West China Hospital, Sichuan University, Chengdu, China. All experimental animals were cared for in accordance with the Guide for Care and Use of Laboratory Animals.

Plasma and liver homogenate was sampled from naïve healthy female Beagle dogs (3 female dogs for plasma sampling, 1 female dog for liver homogenate sampling). A total of 54 naïve healthy Beagle dogs (half males, half females; 9–12 kg in weight) were prepared for the test of 50% effective dose. After a washout period of at least 3 weeks, 30 Beagle dogs (half males, half females; 9–12 kg in weight) were prepared for pharmacodynamic testing procedures. After a washout period of at least 3 weeks, 26 female dogs (10–12 kg) were enrolled in the adrenal function test. All animals were housed individually and fed at 4 pm once per day. Water was provided *ad libitum*. All the experimental procedures were performed in the light phase, and animals were randomly allocated into each group.

Drugs: ET-26, ET-42, MOC-etomidate and CPMM were all prepared in our laboratory. MOC-etomidate was synthesized using a previously reported method (Cotten et al., 2009). CPMM was synthesized using a previously reported method (Husain et al., 2012). ET-26 was synthesized using the indicated synthesis route (Fig.1 b.). ET-42 was synthesized using the indicated synthesis route (Fig.1 c.). Spectral data for ET-26, ET-42, MOC-etomidate and CPMM are provided as supplementary data. Also see supplementary data for further details on the preparation of ET-26 and ET-42. All drugs were dissolved in dimethyl sulphoxide (DMSO) immediately prior to use.

2.2. In Vitro Studies: Metabolite Stability in the Plasma and Liver Homogenate

The whole blood was sampled from one naïve healthy female Beagle dog and the blood samples were mixed with heparin immediately after sampling. Liver tissue was taken promptly from a naïve humanely killed female dog.

A 25% liver homogenate was prepared using a 0.1 mol/L phosphate-buffered saline solution (pH 7.4). Plasma samples derived from the

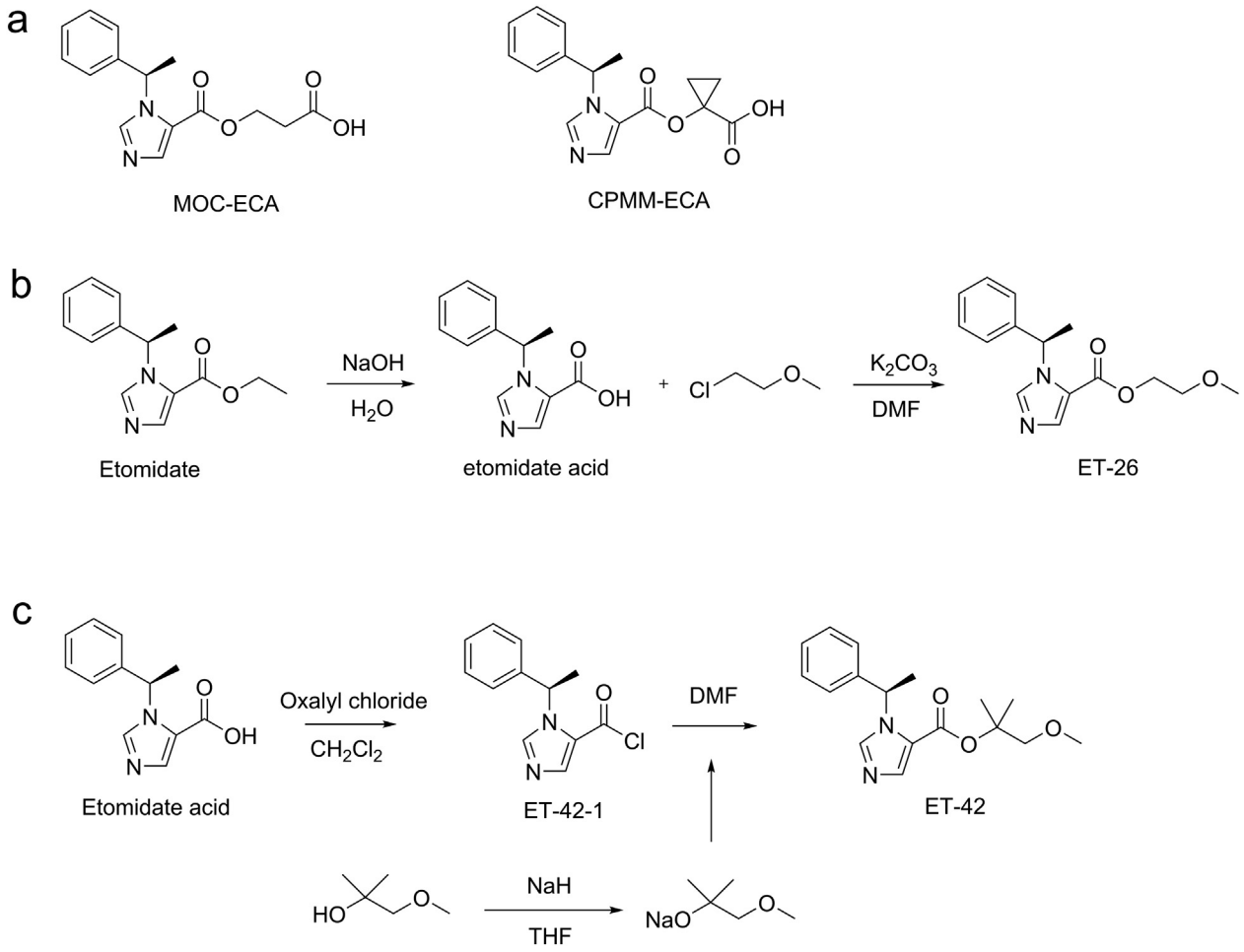

Fig. 1. The primary metabolites of (a). MOC-etomidate and CPMM, the synthesis routes of (b). ET-26, and (c) ET-42. MOC-etomidate: methoxycarbonyl etomidate; CPMM: cyclopropylmethoxycarbonyl etomidate.

blood and liver homogenate were divided into five samples, each with a 4-mL volume. Each sample of plasma or liver homogenate was warmed to 37 °C for 15 min; then, Etomidate or related analogues, including ET-26, ET-42, MOC-etomidate and CPMM, (10 mg/mL, dissolved in DMSO) were added to the plasma or liver homogenates to obtain a final concentration of 50 μg/mL. After incubation for 0, 5, 10, 15, 20, 30, 40, 60 and 120 min, 100 μL of the incubated plasma or liver homogenate was removed, and the metabolic reaction was quenched using acetonitrile (300 μL). All samples were centrifuged at 20,000g for 15 min and analyzed using high-pressure liquid chromatography (HPLC) (Agilent 1100 series, Agilent Technologies, USA).

Prototype drugs were determined by an isocratic elution chromatography. The ultraviolet (UV) absorbance detector was set at 242 nm for detection of the prototype drugs. Gradient-elution chromatography was used to determine the presence of etomidate acid, and the UV absorbance detector was set at 232 nm. The limits of quantification in plasma for ET-26, ET-42, etomidate, MOC-etomidate, CPMM and etomidate acid were 1.04 μg/mL, 1.02 μg/mL, 1.06 μg/mL, 1.05 μg/mL, 1.11 μg/mL and 2.22 μg/mL, respectively. The limits of quantification in liver homogenate for ET-26, ET-42, etomidate, MOC-etomidate, CPMM and etomidate acid were 1.06 μg/mL, 1.08 μg/mL, 1.09 μg/mL, 1.07 μg/mL, 0.5 μg/mL and 2.27 μg/mL, respectively. The detailed method of drug assay was provided as supplementary data.

2.3. In Vivo Studies

2.3.1. Pharmacodynamic evaluation

54 naïve healthy Beagle dogs (half males, half females; 9–12 kg in weight) were selected at random. The dogs were catheterized in the cephalic vein with a vein IV catheter (20 gauge, 19 mm). The 50% effective dose (ED_{50}) for loss of righting reflex (LORR) was established using an up-and-down sequential allocation design: one Beagle dog was administered an initial dose of a test drug according to the weight of the dog, and any incidences of LORR were recorded; if LORR occurred, a lower dose was then administered to the next dog; if LORR did not occur, the higher dose was then administered to the next dog; when LORR happened to a dog and did not happen to the next dog, one crossover was recorded, the test continued until a total of three crossovers were obtained, and an ED_{50} was then calculated based upon these observations (Dixon, 1991).

The initial doses of all the drugs used were based on the findings of the preliminary experiment. ET-26, ET-42, etomidate, MOC-etomidate and CPMM dissolved in DMSO were all administered through the intravenous catheter, followed by a 1-mL saline flush. After the injection, the timing procedure was started. Maintenance of LORR for longer than 30 s was considered to indicate anaesthesia (Zhang et al., 2014). The onset time was 0 min in all experiments because all drugs resulted in LORR immediately after administration.

After ED_{50} of every drug was obtained, five groups of healthy dogs (six dogs per group, half males and half females) were used for investigation of the pharmacodynamic characteristics of ET-26, ET-42, Etomidate, MOC-ET and CPMM. Each agent was intravenously administered at a dose of twice the ED_{50}. The corneal reflex and the righting, or eyelash reflex, were measured to indicate sedation and anaesthesia. The timing of LORR was regarded as the duration of anaesthesia; the time from recovery of righting reflex to free walking was regarded as the recovery time. The duration of anaesthesia and the recovery time were recorded by a blinded observer. Muscle tremor is another major side effect of etomidate; we thus recorded the incidence of muscle tremor in each group, using a criterion of a duration of muscle tremor >30 s during anaesthesia. Body temperatures of all animals were maintained at 36–38 °C during anaesthesia.

2.3.2. Adrenocortical Function Test

A total of 26 adult female dogs were randomly divided into six groups, with 6 dogs in the CPMM treatment group and 4 dogs in all other groups, resulting in a sample number of 4–6. All drugs were administered at a dose of twice the ED_{50}, dissolved in DMSO. DMSO containing no active agent was administered as the control. Cortical hormone concentrations were measured before administration of drugs and 60 min after ACTH stimulation.

Prior to the test, dexamethasone (0.01 mg/kg) was administered to each dog to suppress baseline cortisol and corticosterone levels and to exclude the effects of variable stress responses to handling. Two hours later, the blood samples were obtained for measurement of the baseline serum cortisol and corticosterone concentrations. After sample collection, ET-26, ET-42, etomidate, MOC-ET and CPMM were intravenously administered at a dose of twice ED_{50}, respectively for each group. A total of 250 μg of adrenocorticotropic hormone$_{1-24}$ ($ACTH_{1-24}$) (Sigma-Aldrich Chemical Co., St. Louis, MO) was injected into each dog 15 min after sampling to stimulate cortisol and corticosterone production. The consequent blood sample was collected at 1 h after administration of $ACTH_{1-24}$ (Fig. 2). Blood samples (of a 4 mL volume) were cultured and clotted at 37 °C for approximately 30 min, then centrifuged at 3500 rpm at 4 °C for 10 min. Serum samples were then transferred and frozen at −20 °C, followed by measurement of cortisol and corticosterone levels within 1–2 days. After the thawing process, blood samples were centrifuged at 16,000 rpm, at 4 °C for 10 min, and were then quantified using an enzyme-linked immunosorbent assay (ELISA kit; Enzo Life Science) and a 96-well plate reader. A diagnosis of suppression of adrenal function was usually made following the detection of an impaired cortisol response to corticotrophin (Cotten et al., 2010; Marik et al., 2008). The methods used to investigate dog adrenal function were optimized and adapted according to several previously published reports (Maze et al., 1991; Foster et al., 2000; Pessina et al., 2009).

2.4. Statistical Analysis

Data were analyzed using the Dixon–Mood method to derive the median effective doses [$ED_{50} = x_0 + i(A/N \pm 0.5)$] with a 95% confidence interval (CI). *In vitro* data on the stability of ET-26, ET-42, etomidate, MOC-etomidate and CPMM, and the concentrations of cortical hormones are presented as mean ± standard deviation (SD), and the *N* denotes the number of dogs in each group. Data on rates of metabolism were analyzed using a one-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc test. A Kruskal–Wallis test, followed by a Mann–Whitney *U* test was applied to assess differences in the duration and recovery time observed in the *in vivo* tests. Plasma cortisol and corticosterone concentrations after compound or vehicle administration were linearized, log-transformed and compared using a one-way ANOVA followed by a least-significant difference (LSD) or Tamhane multiple-comparison test. A P-value < 0.05 was considered statistically significant. All analysis was performed using Statistical Package for Social Sciences (SPSS™) software for Windows, version 21 (SPSS, Chicago, IL, USA). Figure preparation and curve fitting was performed using Origin 8.0 (Origin Lab Corp., Northampton, MA, USA).

3. Results

3.1. Metabolite Stability in Plasma and Liver Homogenate

ET-26, ET-42 and etomidate were stable in plasma samples, even after a 2-h incubation. MOC-ET can be metabolized 25.23 ± 1.78% and CPMM can be metabolized 19.83 ± 0.79% in plasma in 2 h (Fig.3 a). The primary metabolites of MOC-ET and CPMM were MOC-ECA (Ge et al., 2012a, b) and CPMM-ECA (Campagna et al., 2014), no etomidate acid was detected at any point during the 2-h incubation period.

ET-42 was stable in liver homogenate samples, even after a 2-h incubation period. The percentages of metabolized ET-26 and etomidate were 19.02 ± 0.06% and 10.74 ± 1.71% in liver homogenate after a 2-h incubation, and the rate of decomposition of ET-26 was significantly greater than that of etomidate ($P < 0.05$). CPMM was undetectable,

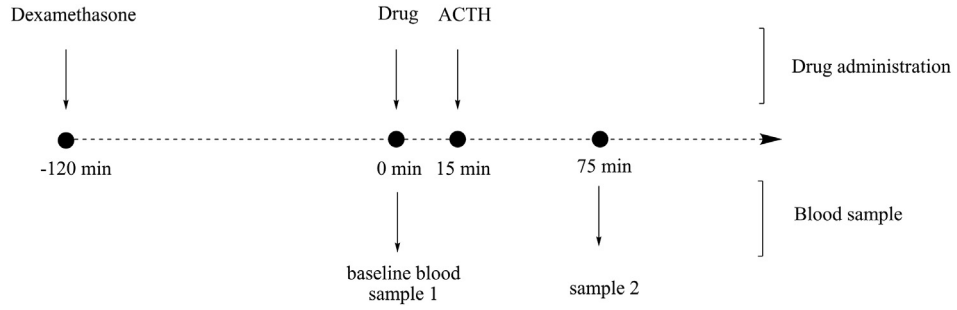

Fig. 2. The experimental protocol of the cortical hormone stimulation test.

and MOC-etomidate was barely detectable (1.54 ± 0.06% of the concentration at the start of the incubation) after a 10-min incubation in liver homogenate (Fig.3 b.), indicating that CPMM and MOC-etomidate are much more rapidly degraded than the other agents tested (*Vs.* ET-26, $P < 0.05$). When incubated in plasma, no etomidate acid was detected after a 2-h incubation of MOC-etomidate or CPMM in liver homogenate.

Etomidate acid was found to be the primary metabolite of both ET-26 and etomidate: the concentrations of etomidate acid were 8.79 ± 0.37 μg/mL and 3.24 ± 0.16 μg/mL in the ET-26 and etomidate groups, respectively. The initial concentration of both ET-26 and etomidate in liver homogenate was 50 μg/mL. Theoretically, 19.02% of this concentration of ET-26 and 10.74% of this concentration of etomidate would result in etomidate acid concentrations of 7.50 μg/mL and 4.75 μg/mL, respectively. The measured concentrations of etomidate acid were found to conform approximately with these calculated values. Therefore, we are able to confirm that our measurements of etomidate acid production are consistent with our measurements of ET-26 and etomidate degradation.

3.2. LORR Test

DMSO, the solvent control, had no anaesthetic or adverse effects. The ED_{50} of ET-26, ET-42, etomidate, MOC-etomidate and CPMM for LORR in dogs was 1.44, 0.72, 0.43, 23.12, and 0.59 mg/kg, respectively. Pharmacodynamic characteristics of ET-26, ET-42, etomidate, MOC-etomidate and CPMM were also investigated (Table 1).

3.3. Equivalent Study

Doses corresponding to twice the ED_{50} were given to induce LORR. All the dogs were anaesthetized during injection; therefore, the onset time for the effects of all agents was 0 min. The duration of anaesthesia was 3.21 ± 0.90, 7.94 ± 0.41, 5.36 ± 1.91, 2.43 ± 0.35 and 2.21 ± 0.42 min in the ET-26, ET-42, etomidate, MOC-etomidate and CPMM groups, respectively. The duration of the recovery time was 1.84 ± 0.82, 9.67 ± 0.50, 1.78 ± 0.93, 5.08 ± 1.09 and 1.21 ± 0.53 min in the ET-26, ET-42, etomidate, MOC-etomidate and CPMM groups, respectively (Table 1). No significant differences were observed in the duration of anaesthesia in the ET-26 group compared with that of the MOC-etomidate and CPMM groups ($P = 0.655$ *versus* MOC-etomidate, and $P = 0.426$ *versus* CPMM). The mean duration of anaesthesia in the ET-26, CPMM and MOC-etomidate groups was significantly shorter than that of the ET-42 group ($P < 0.05$). No significant differences in the mean duration of recovery time were observed in the ET-26 group compared with that of the etomidate and CPMM groups ($P = 1.000$ *versus* etomidate, $P = 0.394$ *versus* CPMM). The mean duration of recovery time in the MOC-etomidate and ET-42 groups was significantly longer than that of the ET-26, etomidate and CPMM groups ($P < 0.05$). In addition, the incidence of muscle tremor in the ET-26 group was lower than that of the other four groups (Table 1).

3.4. Adrenocortical Function Test

The effects of ET-26, ET-42, etomidate, MOC-etomidate and CPMM on adrenocortical function were assessed using measurements of serum cortisol and corticosterone concentrations.

The baseline of serum cortisol concentrations was 347.42 ± 83.19 pg/mL, 368.66 ± 204.38 pg/mL, 251.55 ± 75.30 pg/mL, 269.76 ± 97.26 pg/mL, 248.28 ± 44.73 pg/mL and 308.12 ± 290.86 pg/mL in the ET-26, ET-42, etomidate, MOC-etomidate, CPMM and cntrol groups, respectively. After ACTH stimulation, serum cortisol concentrations was 3493.84 ± 1558.09 pg/mL, 3657.97 ±

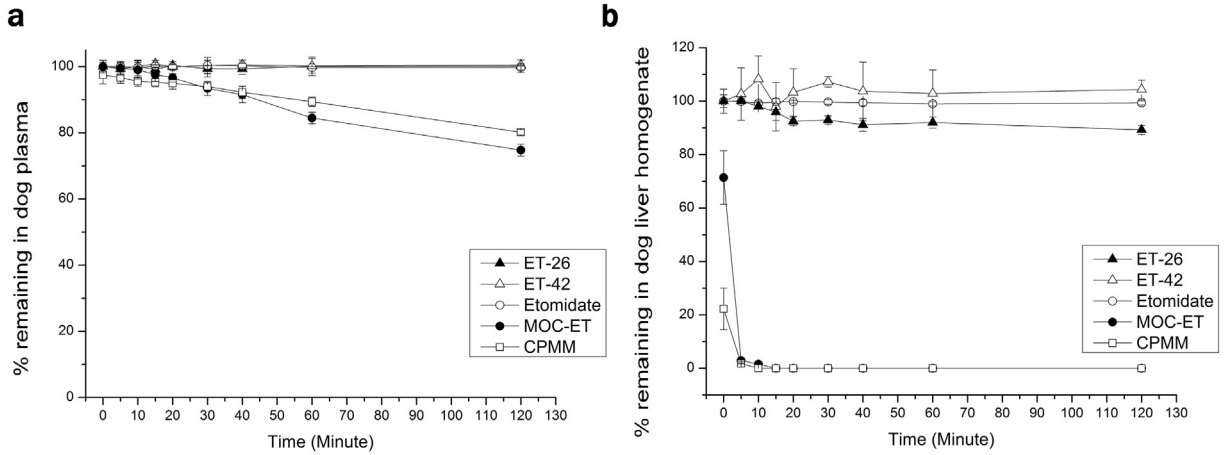

Fig. 3. *In vitro* 2-h decomposition curves of the five test drugs in (a). plasma and (b). liver homogenate.

Table 1
Pharmacodynamic characteristic of the 5 drugs in Beagle dogs ($N = 6$).

Drug	ED_{50} (with 95% CI, mg/kg) (Atucha et al., 2009)	Dose ($2ED_{50}$) mg/kg	Onset time (Bovill, 2006) min	Duration time (Campagna et al., 2014) min	Recovery time (Cotton et al., 2008) min	Incidence of muscle tremor (Cotten et al., 2009)
ET-26	1.44 (1.63–1.27)	2.88	0	3.21 ± 0.90	1.84 ± 0.82	2/6
ET-42	0.72 (0.75–0.69)	1.44	0	7.94 ± 0.41	9.67 ± 0.50	3/6
Etomidate	0.43 (0.48–0.39)	0.86	0	5.36 ± 1.91	1.78 ± 0.93	4/6
MOC-ET	23.12 (26.19–20.40)	46.24	0	2.43 ± 0.35	5.08 ± 1.09	6/6
CPMM	0.59 (0.60–0.57)	1.18	0	2.21 ± 0.42	1.21 ± 0.53	6/6

1: The ED_{50} was determined by the up-and-down method (Dixon, 1991).
2: Onset time denotes the time from injection of the drug to disappearance of the righting reflex.
3: Duration time denotes the time from disappearance of the righting reflex to recovery of the righting reflex.
4: Recovery time denotes the time from recovery of the righting reflex to free walking.
5: Criterion of muscle tremor was that the tremor time of extremities or trunk was >30 s during anaesthesia.

1533.68 pg/mL, 436.79 ± 109.52 pg/mL, 357.29 ± 104.36 pg/mL, 5450.39 ± 1947.61 pg/mL and 8596.66 ± 3315.59 pg/mL in the ET-26, ET-42, etomidate, MOC-etomidate, CPMM and cntrol groups, respectively.

The baseline of serum corticosterone concentrations was 478.60 ± 195.37 pg/mL, 247.10 ± 165.10 pg/mL, 464.13 ± 293.78 pg/mL, 193.84 ± 176.60 pg/mL, 241.38 ± 185.08 pg/mL and 150.80 ± 49.52 pg/mL in the ET-26, ET-42, etomidate, MOC-etomidate, CPMM and cntrol groups, respectively. After ACTH stimulation, serum corticosterone concentrations was 3088.19 ± 1886.67 pg/mL, 2197.99 ± 884.99 pg/mL, 609.50 ± 73.34 pg/mL, 468.21 ± 208.30 pg/mL, 3335.25 ± 1943.27 pg/mL and 4380.09 ± 1651.06 pg/mL in the ET-26, ET-42, etomidate, MOC-etomidate, CPMM and cntrol groups, respectively.

No significant differences in baseline cortisol and corticosterone concentrations were observed across all groups (cortisol: $P = 0.731$; corticosterone: $P = 0.431$). Serum cortisol concentrations in the CPMM, ET-26 and ET-42 groups were significantly higher than those of the etomidate and MOC-etomidate groups at 1 h after ACTH stimulation ($P < 0.05$). No significant differences in serum cortisol concentrations at 1 h after ACTH stimulation were observed between the control group and the ET-26, ET-42 and CPMM groups ($P = 0.755$ *versus* CPMM, $P = 0.115$ *versus* ET-26, $P = 0.136$ *versus* ET-42) (Fig.4 a.). Serum corticosterone concentrations in the ET-26, ET-42 and CPMM groups at 1 h after ACTH stimulation were significantly higher than those of the etomidate and MOC-etomidate groups ($P < 0.05$). No significant differences were observed in serum corticosterone concentrations at 1 h after ACTH stimulation in the ET-26, ET-42 and CPMM groups compared with those of the control group ($P = 0.674$ *versus* CPMM, $P = 0.389$ *versus* ET-26, $P = 0.199$ *versus* ET-42) (Fig.4 b.). These results indicate that, unlike etomidate and MOC-etomidate, ET-26, ET-42 and CPMM could relieve cortex suppression in Beagle dogs.

4. Discussion

Etomidate has a potent inhibitory effect on 11-β hydroxylase enzymes; however, its metabolite, etomidate acid, has an inhibitory potency approximately 1/120,000 of that of etomidate (Zolle et al., 2008), indicating almost no inhibitory effect. Intravenous etomidate results in obvious inhibition of cortical hormone synthesis, owing to slow *in vivo* metabolism. To overcome this adverse effect of etomidate, a number of promising etomidate softdrug have been developed (Cotten et al., 2009; Husain et al., 2012; Pejo et al., 2012b). These molecules are rapidly metabolized *in vivo*; therefore, the prototype drug can be rapidly eliminated from the body. These prototype drugs are unable to suppress cortical hormone synthesis for a long period of time after drug withdrawal.

However, these softdrug are generally not metabolized primarily to etomidate acid. Etomidate softdrug are rapidly metabolized *in vivo* by hydrolytic enzymes located throughout the body. Owing to their considerable polarity, the primary metabolites generated by the hydrolytic enzymes cannot easily cross the blood–brain barrier, and can accumulate in both the brain and the periphery. If these primary metabolites have some inhibitory effects on the CNS, they will affect the quality of the patient's postoperative recovery; if the primary metabolites have some cortical hormone inhibitory activity, they may produce obvious

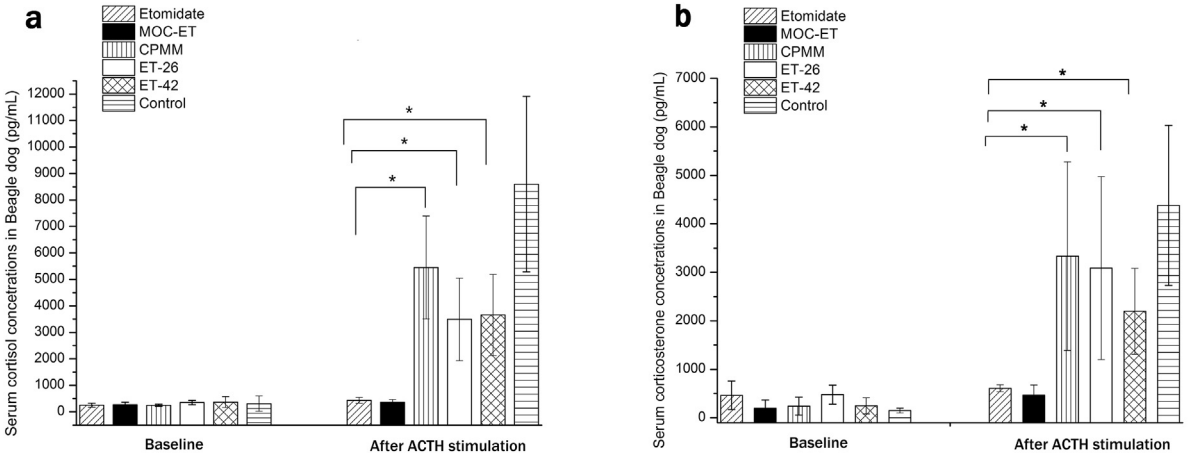

Fig. 4. Comparisons of (a). ACTH-stimulated serum cortisol concentrations, and (b). ACTH-stimulated serum corticosterone concentrations. ACTH; adrenocorticotropic hormone.

cortical hormone suppression after drug withdrawal. For example, MOC-ECA has been confirmed to have an inhibitory effect on both the CNS and on cortical hormone (Ge et al., 2012a, b).

In this study, MOC-etomidate and CPMM were rapidly metabolized in both plasma and liver homogenate samples. After incubation in liver homogenates for 15 min, MOC-etomidate and CPMM are almost completely metabolized. Interestingly, no etomidate acid was detected even after a 2-h incubation of MOC-etomidate or CPMM in either plasma or liver homogenates. MOC-etomidate can be hydrolyzed *in vivo* to MOC-ET-ECA (Cotten et al., 2009), indicating that MOC-ET-ECA cannot be rapidly hydrolyzed to etomidate acid. Despite MOC-etomidate being rapidly metabolized, ACTH stimulation did not result in an increase in cortical hormone levels 75 min after administration of MOC-etomidate, indicating that cortical hormone synthesis continued to be inhibited. We speculate that this prolonged inhibition is probably caused by accumulation of MOC-ET-ECA. Similar to MOC-etomidate, CPMM can also be rapidly hydrolyzed during *in vitro* incubation in liver homogenate for 10 min. CPMM-ECA, as a primary metabolite of CPMM, could not be rapidly metabolized to etomidate acid. However, no significant suppression of corticosteroid levels was observed, suggesting that CPMM-ECA does not suppress circulating cortical hormone levels. These results demonstrate that knowledge of the major primary metabolites is a very important consideration in the design and testing of etomidate analogues.

The anaesthetic effects of etomidate originate from its ability to activate $GABA_A$ receptors (Siegwart et al., 2002), while the suppression of cortical hormone levels is a result of inhibition of 11-β hydroxylase enzymes (Vanden Bossche et al., 1984). Investigations of the structure–activity relationships of etomidate indicate that the structure of the imidazole carboxylic acid ester side chain is important for both of these effects (Atucha et al., 2009). Therefore, specific modifications of these ester side chains might enable the anaesthetic effects of etomidate to be retained, with a reduction in the extent of suppression of cortical hormone levels. If etomidate acid is the primary metabolite of such molecules, this would suggest that neither the prototype agents, nor its metabolite, are likely to inhibit cortical hormone biosynthesis.

With these previous findings in mind, the properties of ET-26 and ET-42, which are etomidate analogues with different ester side chains, were investigated in this study. The results of this study show that ET-26 and ET-42 produce definite and reversible anaesthetic effects, and that the anaesthetic efficacy of ET-26 is close to one-third that of etomidate, and the efficacy of ET-42 is close to half that of etomidate. In addition, we observed that the incidence of muscle tremor in the ET-26 group is significantly lower than that of the other four groups. Etomidate acid, as observed in liver homogenates, was found to be a major metabolite of ET-26. Furthermore, ET-26 and ET-42, similar to etomidate, was found to be much more slowly metabolized than both MOC-etomidate and CPMM.

ET-26, ET-42 and CPMM, unlike etomidate and MOC-etomidate, did not inhibit the response to ACTH stimulation at 1 h after administration. Both ET-26 and ET-42 were not rapidly metabolized when incubated in plasma or liver homogenate, suggesting that the lack of suppression of serum corticosteroid levels is likely to be a characteristic of the prototype drug itself. This feature of ET-26 and ET-42 is similar to that of carboetomidate, which was generated by substitution of the nitrogen atom of the imidazole ring of etomidate with a methylene group (Cotten et al., 2010). The anaesthetic potency of carboetomidate is approximately 1/7 that of etomidate; thus, compared with carboetomidate, ET-26 and ET-42 are both more potent anaesthetics.

A high level of anaesthetic potency and a lack of inhibition of cortisol synthesis are the main requirements for the development of 'better etomidate'. ET-26 and ET-42 were selected from dozens of analogues designed using our synthesis strategy. We consider the number of new compounds to be far from sufficient, with considerable opportunity available to expand the range of candidate compounds for screening. In addition, concerns remain as to whether ET-26 and ET-42 have the wide therapeutic index and cardiovascular stability of etomidate. These unresolved concerns will form the basis of subsequent research.

5. 5. Conclusions

In this study, ET-26 and ET-42 were demonstrated to be highly potent anaesthetics. ET-26, similar to etomidate, is slowly metabolized to etomidate acid in liver homogenates. ET-42 is more slowly metabolized than both ET-26 and etomidate. Despite this slow metabolism, both ET-26 and ET-42 have no significant effects on adrenal function. Our results indicate that etomidate derivatives that are not rapidly metabolized and that have pharmacologically inactive metabolites (such as etomidate acid) might provide an alternative to etomidate, with a reduced risk of adverse effects on corticosteroid levels.

Abbreviations

ACTH	adrenocorticotropic hormone
ET-26	methoxyethyl etomidate
ET-42	methoxy-2-methylpropan etomidate
MOC-ET	methoxycarbonyl etomidate
MOC-ET-ECA	methoxycarbonyl etomidate acid
CPMM	cyclopropyl-methoxycarbonyl etomidate
CPMM-ECA	cyclopropyl-methoxycarbonyl etomidate acid

Conflicts of Interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary Data

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