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ORIGINAL ARTICLE

Evaluation of Etomidate as an Intravenous Anesthetic Drug in Dogs: Using Midazolam and Methocarbamol in Premedication

Moosa Javdani[1*], Ali Hashemi[1], Amin Bigham-Sadegh[1], Zahra Nikousefat[2]

[1] *Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.*

[2] *Department of Clinical Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran.*

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Abstract

Objective- The aim of this study was to evaluate etomidate as an injectable anesthetic in dogs and to use midazolam and methocarbamol in premedication.
Design- Experimental Study.
Animals- Fourteen native young female dogs
Procedure- Dogs were randomly allocated to one of two groups to receive midazolam (0.5 mg/kg; MiE group) or methocarbamol (20 mg/kg; MeE group) 5 minute before etomidate (2 mg/kg) administered intravenously. In addition to recording anesthesia plan times, some physiological and hematobiochemical parameters were also measured at different times in the two groups.
Results- It was found that there was no significant difference between the two groups at both induction and recovery time. Heart rate, respiratory rate, and rectal temperature changes in the MiE group were noticeably fluctuating, unlike the MeE group. There was a significant difference in the values of hematocrit, total protein, glucose, and cortisol between the two groups at some times. Significant increase in ALT and AST activity was observed in MeE group compared to MiE group at some times.
Conclusion and Clinical Relevance- Intramuscular administration of methocarbamol such as midazolam prior to induction of anesthesia by etomidate in dogs, while providing similar anesthesia and recovery time, has minimal cardiopulmonary effects and hematobiochemical changes in dogs under anesthesia.

* Correspondence to: Moosa Javdani, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran. E-mail: javdani59@gmail.com

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1. Introduction

Choosing a proper and safe anesthesia regime is always an integral part of many surgeries. In a balanced anesthesia, it is always attempted to exert minimal adverse effects on various organs of the body, especially the respiratory and cardiovascular system. Etomidate is a short-acting anesthetic that may be prescribed for induction of anesthesia and sedation in some minor surgeries and therapeutic interventions, including the treatment of joint dislocation and patient intubation.[1] Etomidate induces anesthesia by enhancing GABAergic inhibitory neurotransmitter activity.[2] It has been suggested that this fast-acting drug has the least cardiovascular complications and hypotension and is therefore a good agent for induction of anesthesia.[3] Respiratory system suppression following administration of etomidate was negligible. In addition, myocardial and brain protection from ischemia has been reported due to a lack of release of histamine after etomidate administration.[1] It is used in patients with head injury because it maintains normal blood pressure by lowering intracranial pressure.[4] It has been found that etomidate induces rapid anesthesia induction and recovery in dogs and because it has minimal cardiovascular changes, even in hypovolemic dogs, it is an appropriate drug for induction and maintenance of anesthesia using total intravenous anesthesia (TIVA) technique. But its administration can lead to excitation, muscle contractions, pain at the injection site, nausea, and respiratory apnea at the time of induction of anesthesia in the dog. Therefore, premedication is recommended before induction of anesthesia with etomidate. Midazolam as a benzodiazepine drug with a muscle relaxant and sedative effects and also with minimal cardiovascular complications is used for pre-anesthesia and anesthesia in the veterinary field.[5-7] Methocarbamol is a central-acting muscle relaxant that relieves muscle contractions by suppression the central nervous system. The mechanism of its action is not well understood, but it appears to induce its effects by inhibiting carbonic anhydrase.[8] The analgesic property has been described for methocarbamol. In addition, it has been suggested that this drug may relax the skeletal muscles by blocking the spinal cord through polysynaptic inhibition. It is therefore prescribed in the treatment of many musculoskeletal pains, post-operative pain, severe muscle spasm, and neurological disorders.[9] The central muscle relaxant effect of midazolam and Methocarbamol, in addition to their useful properties, may justify their beneficial use before administering etomidate to dogs. So, in the current investigation, intramuscular administration of midazolam and Methocarbamol was evaluated before intravenous administration of etomidate in dogs.

2. Materials and Methods

Ethical and executive aspects of this study were approved by the Research Council of the Department of Veterinary Clinical Sciences (170/1073). A total of 14 female indigenous dogs (5-7 months old) weighing 5.1-2.23 kg were considered for the present study. Animals were transferred to the Animal House Center of Shahrekord University at least two weeks before the study began and the animal's health status was confirmed by clinical examination, complete blood count (CBC), and total protein (TP). In addition, they received standard antiparasitic treatment. The animals were housed in single cages with free access to water and fed twice daily. Dog feeding was prohibited 12 hours before each experiment, but water was freely available during the study. Dogs were randomly divided into two equal groups receiving midazolam-etomidate (MiE group) and methocarbamol-etomidate (MeE group). After transferring the dogs to the experiment site, the animals were adapted to the new environment for 30 minutes and their behavior was monitored. After measuring the heart rate (HR), respiratory rate (RR), and rectal temperature (RT) of animals and routine cephalic venous catheter insertion, in the MiE group midazolam (0.5 mg/kg; Midamax®, Tehran Chemie,

Iran) and in the MeE group methocarbamol (20 mg/kg; Relaxin®, Sina Daroo, Iran) were administrated intravenously.[10]

Five minutes after prescribing of premedication, etomidate (2 mg/kg; Hypnomidate®, Piramal, UK) was injected intravenously, and some physiological parameters such as RT, RR, and HR were recorded from time 0 (before midazolam and methocarbamol injection) and at 5 min intervals until complete recovery of the animals.

Clinical Evaluation

Following administration of etomidate, the time between administration of this drug and the loss of the pharyngeal reflex (the possibility of tracheal intubation) were recorded as the induction time of anesthesia, and the time interval between the return of the pharyngeal reflex to the creation of the righting reflex (return of the animal to the external position) was recorded in the animals as recovery time. So, the time between loss and return of the pharyngeal reflex was registered as the duration of anesthesia (maintenance time).

Hematobiochemical Assessment

Intravenous blood sampling was performed at time 0, 130, 180 minute, and 24 and 48 hours after any drug injection to measure hematocrit (HCT), and cortisol hormone. For this purpose, the collected samples were sent to the laboratory with laboratory tubes containing and without EDTA. The cortisol was measured by competitive ELISA using a commercial monobind cortisol assay kit. Blood glucose was measured manually using the Pars Azmoon kit. Additionally, total plasma protein was measured by Biuret method using commercial technique (Pars Azmoon kit, Pars Azmoon Inc., Tehran, Iran).[11] Modified Reitman Frankel method was used to measure alanine transferase (ALT) activity.[12] In addition, the Hitachi Auto-analyzer (Model 717, Japan) was used to measure the activity of aspartate aminotransferase (AST) by the photometric method. HTC was determined by micro-hematocrit method.[13]

Histopathological Analysis

The liver, kidney, and testis, were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, the tissues were dehydrated in graded ethanol, clearing in Xylol, loading in paraffin wax and sectioning at about 5-6 µm. The resulting samples were stained using hematoxylin and eosin (H&E) and observed by light microscopy (Olympus, BX60). For a semi-quantitative comparison of the structural changes, the abnormalities in the tissue sections were graded from 0 (normal structure) to 3 (severe pathological changes).

Statistical Analysis

The recorded data was reported as Mean (±SEM) using SPSS statistical software version 22 and Graphpad PRISM version 7. Comparison of parameters of induction, maintenance and recovery time of anesthesia in two groups of MiE and MeE was performed by statistical Independent Student's t-tests. The mean (± SEM) of HR, RR, RT, HTC, and cortisol hormone level were compared between two groups with ANOVA test at different times in each group. Significant differences were reported at the $p < 0.05$ level.

3. Results

Results related to anesthesia times are presented in Table 1. It was observed that there was no significant difference between the two groups at both induction and recovery times ($p > 0.05$). Comparison of mean (± SEM) of HR at different times did not show any significant difference between the two groups. In-group assessment of this parameter in the MeE group, changes observed at various times were not significantly different from each other ($p > 0.05$). Whereas in the MiE group the HR parameter showed a significant increase in time 5 and again after its decrease at time 10 and 15, increased again at time 20 ($p <$

0.05). In other words, HR changes in this group were noticeably fluctuating, unlike the MeE group (Figure 1).

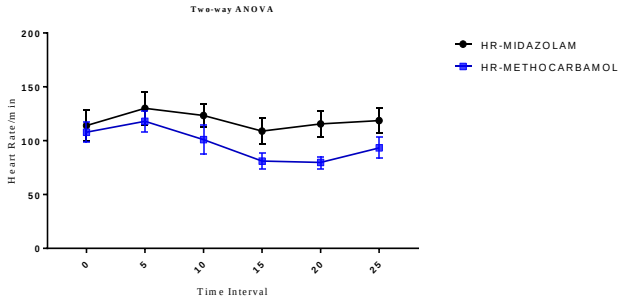

Figure 1. HR parameter changes during anesthesia in two groups of MiE and MeE

Comparison of the mean (± SEM) of RR between the two groups showed a significant increase in this parameter at time 10 and in the MiE group ($p < 0.05$). The intra-group analysis of this parameter in the MiE group showed a significant increase at time 5 and then considerable decrease and finally a significant increase at time 20 ($p < 0.05$). But in the MeE group there were no significant changes at different times (Figure 2).

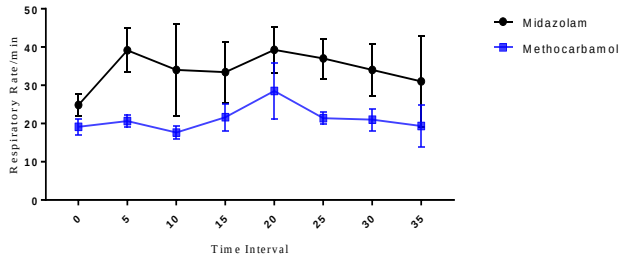

Figure 2. RR parameter changes during anesthesia in two groups of MiE and MeE

Comparison of the mean (± SEM) of RT between the two analyzed groups showed a significant increase in this parameter at time 5 and in the MiE group compared to the MeE group ($p < 0.05$). In the MeE group there were no significant changes in RT at different times. But, only at time 5 of the MiE group a significant increase in the mean of this parameter was recorded ($p < 0.05$).

Table 2 shows the mean (± SEM) comparison of some hematobiochemical parameters. Intergroup analysis of HCT showed a significant increase in HCT at 180 minutes and 48 hours in the MeE compared to the MiE ($p < 0.05$). But in-group analysis of this parameter showed no considerable difference at different times ($p > 0.05$).

The results showed that the TP in the MeE group at the times of 120, 180 minutes and 24 hours after anesthesia significantly decreased compared to the MiE group ($p < 0.05$). In both groups, a significant decrease in mean of this parameter was observed at times 30, 120, and 180 ($p < 0.05$).

Comparison between groups showed a significant increase in the mean glucose parameter at time 120 and 180 of study in MeE group compared to MiE. In both groups, this marker increased significantly at time 5 of anesthesia. While there was no significant change in the MiE group, there was again a significant increase in mean glucose at 120 MeE ($p < 0.05$).

Statistical comparison of the mean cortisol between the two groups revealed that there was a significant difference at 5, 30, and 120 minutes of study ($p < 0.05$). The mean (± SEM) evaluation of cortisol in both groups showed a significant increase at time 5 and a significant decrease at time 30 anesthesia ($p < 0.05$). Subsequently, the upward trend of this parameter was observed up to time 180.

Significant increase in ALT activity was observed in MeE group compared to MiE group at times 5, 30, and 180 studies ($p < 0.05$).

Significant increase in ALT activity at time 30 was observed in the MiE group. Then, at 120 and 180 minutes and 24 hours after anesthesia its activity decreased significantly ($p < 0.05$). Considerable increase in the activity of this enzyme was observed in MeE group at 5 and 30 minutes after anesthesia. And of course a significant decrease in subsequent ALT activity was observed (Table 2).

Comparison of AST activity between two groups showed a significant increase in AST at 24 and 48 hours ($p < 0.05$). There was a significant increase in the mean (± SEM) of AST in the MiE group only at time 30 of the study and then recorded without any significant changes. However, in the MeE group, only time 24 of the studies showed a significant increase in the activity of AST (Table 2).

Table 1. Comparison of mean (± SEM) anesthesia times data between two groups of MeE and MiE

Group	Induction time (sec)	Maintenance time (min)	Recovery time (min)
MeE	12.5 ± 2.5	30.75 ± 9.66	30.00 ± 11.65
MiE	11.5 ± 2.8	27.72 ± 12.62	27.14 ± 11.65
***p* value**	0.55	0.53	0.057

MeE: methocarbamol-etomidate group; MiE: midazolam-etomidate group

Table 2. Comparison of mean (± SEM) of some hematobiochemical parameters in in both MiE and MeE groups.

		Time						
Parameter	Group	0	5	30	120	180	24	48
HTC (L/L)	MiE	42.1±1.58	40.1±1.71	40.5±1.82	41.14±1.0	41.17±0.74	43.25±1.05	43.33±2.36
	MeE	44.75±1.62	43.75±1.73	41.71±1.57	44.63±1.37	45.38±1.18	47.2±2.25	49.13±1.16
	p value	0.27	0.20	0.63	0.07	0.02	0.09	0.03
TP (g/dl)	MiE	6.1 ± 0.41	6.21 ± 0.18	4.67 ± 0.17	3.87 ± 0.23	5.34 ± 0.19	6.91 ± 0.12	6.59 ± 0.51
	MeE	5.9 ± 0.35	6.06 ± 0.37	4.81 ± 0.27	2.85 ± 0.27	3.73 ± 0.23	5.36 ± 0.24	6.26 ± 0.38
	p value	0.64	0.71	0.67	0.01	0.00	0.00	0.62
Glucose (mg/dl)	MiE	79.14 ±1.62	128.6 ±5.45	74.43±2.44	81.71±2.82	83.63 ± 1.7	85.25±1.78	87.38±3.32
	MeE	80.43 ±1.60	127.1 ±4.04	88.71±3.52	96.14 ±3.32	87.29 ±0.86	81.14±1.03	81.29±1.71
	p value	0.58	0.84	0.01	0.01	0.09	0.08	0.14
Cortisol (µg/dl)	MiE	4.6 ± 0.47	7.48 ± 0.17	1.32 ± 0.24	3.43 ± 0.32	5.21 ± 0.28	4.64 ± 0.42	4.41 ± 0.37
	MeE	4.1 ± 0.66	6.41 ± 0.27	3.27 ± 0.25	5.43 ± 0.34	5.41 ± 0.31	4.94 ± 0.22	5.03 ± 0.37
	p value	0.55	0.01	0.00	0.00	0.64	0.54	0.26
ALT (U/L)	MiE	55.71 ±4.65	55.57 ±5.82	103.6±4.19	90.86 ±1.53	73.13 ±4.59	56.5 ± 3.59	57.75±2.75
	MeE	55.86 ±7.31	79.57 ±1.81	128.3±7.97	95.14 ±3.69	92.57 ±1.46	59.57±4.16	59.14±5.39
	p value	0.99	0.00	0.02	0.30	0.00	0.58	0.81
AST (U/L)	MiE	38.14 ±2.86	42.69 ±1.23	47.16±1.56	46.66 ±0.54	45.9 ± 0.99	44.63 ± 0.3	44.66±0.75
	MeE	44.29± 0.28	45.19 ±1.26	45.33±0.56	47.56 ±1.63	46.19 ±4.03	56.17 ±0.71	51.5 ± 0.46
	p value	0.054	0.19	0.29	0.61	0.94	0.00	0.00

MeE: methocarbamol-etomidate group; MiE: midazolam-etomidate group; PCV: packed cell volume; TP: total protein; ALT: alanine transferase; AST: aspartate aminotransferase

4. Discussion

Proper premedication is one of the important steps in creating an ideal and balanced anesthesia. Proper premedication and achieving sedation, muscle relaxation, and adequate analgesia can reduce the medication needed to induce anesthesia and increase the quality of induction of anesthesia.[14,15] Therefore, in order to achieve this important result, in addition to the stability of cardiovascular and respiratory parameters, the use and evaluation of various drugs in the premedication and anesthesia stages is crucial.

In the present study it was observed that the time required for complete recovery of the animals was not significantly different between the two groups. It should be noted that in the present study no surgical intervention was performed on the animals and the animals showed no sign of pain at the time of recovery. Animals in the MiE group were found to be standing more comfortably and more balanced during recovery than in the MeE group, probably due to more muscle relaxation and more sedation following methocarbamol administration. When the animal returned from anesthesia (recovery time) in both groups, some side effects such as tremor and muscle contraction were observed. Consistent with this study, Schwarzkopf *et al*. have shown that anesthesia with etomidate induces myoclonus and paddling movements in dogs. They also showed that intravenous injection of midazolam before

induction of anesthesia was effective in reducing myoclonic motility induced by etomidate.[16] However, it has been found that etomidate causes more rough recovery and more adverse reactions during anesthesia than propofol.[17]

There was no significant difference in duration of induction, maintenance, and recovery of anesthesia in any of the study groups. In other words, methocarbamol, such as midazolam, was able to provide muscle relaxation and tranquilization before anesthesia. Benzodiazepines have minimal cardiovascular effects. It is therefore administrated in combination with various anesthetics to reduce the dose of anesthetic and their cardiovascular complications.[18,19] In the present study, there was no significant difference in the HR between the two groups at the same time, but the stability of this parameter in the MeE group was interesting. The present study also confirmed the stability of HR as a physiological parameter in the anesthesia of dogs with methocarbamol. However, in the MiE group given midazolam prior to the etomidate, significant changes in HR were recorded throughout the study. According to the pharmacology of the midazolam, a notable decrease in HR and cardiac output has been demonstrated.[20] It has described the effects of etomidate as a suitable drug for cardiovascular stability.[21]

Because the change in RR can be very different, this parameter alone is not of great value for monitoring anesthetized animal respiratory tract. However, changes in respiratory rhythm can be a sign of changes in the animal's condition. Excessive reduction in RR per minute (bradypnea) can be an indication of the deep level of anesthesia or hypothermia in the anesthetized animal. There are many factors to increase tachypnea and one of the most important is the reduction of the depth of anesthesia.[22] However, the simplest method for partial monitoring of the respiratory tract is to pay attention to the respiratory rhythm of the animal. The results of our study indicated no significant changes in RR in the MeE group. Whereas an increase in RR was seen after midazolam administration. Consistent with this study, Castro *et al*. showed that the use of midazolam decreases the minute volume and the tidal volume and compensates for the increased RR.[23]

The effect of etomidate on respiratory function is somewhat unknown. Some researchers report that it has no effect on respiration, while others have reported that it reduces respiratory rhythm after administration.[2,24,25] It is reported that etomidate increased RR rate in dogs compared to thiamylal.[26] Decrease of body temperature to less than 35 degrees Celsius, called hypothermia, may occur in animals that are anesthetized in a cool environment. Reducing body temperature by one to three degrees is beneficial and can protect the anesthetized animal from a decrease in blood oxygen levels and the possibility of cerebral ischemia.[15] Perk *et al*. reported that during anesthesia with alfentanil etomidate, body temperature decreased following anesthesia due CNS depression and decreased muscle activity, peripheral vasodilation, and its effect on body temperature regulation.[27]

Our results showed that despite increasing RT after midazolam administration, no significant change in this parameter was observed after methocarbamol administration. But it should be noted that midazolam as a benzodiazepine with its central effect can cause hypothermia.[28,29] However, the combination of a benzodiazepine with other anesthetics can produce different results, such as the results of this study. Another study found that there was no significant change in RT at the time of concomitant administration of ketamine and midazolam.[5] The results of the present study showed that HCT did not change significantly after methocarbamol and midazolam administration. In addition, no significant change was observed after etomidate injection. There seems to be limited comprehensive studies on the effect of etomidate on blood cells in dogs.

The study by Ko *et al*. showed the minimal changes in hemodynamic values after infusion of etomidate in dogs.[25]

However, changes in these cells have been measured after administration of various anesthetic compounds. For example, they reported a decrease in hematocrit and an increase in WBC in ketamine-xylazine anesthesia.[30] Or, insignificant changes in red blood cells, leukocytes, hematocrit and hemoglobin occur during ketamine-xylazine administration during dog anesthesia.[31]

In contrast, it is noted that following intramuscular administration of ketamine, the number of leukocytes, hematocrit, erythrocytes, and hemoglobin concentrations decreased markedly.[32] The results of the present study indicated a significant increase in glucose and cortisol after methocarbamol and midazolam administration (time 5). Basically, due to environmental stimuli such as what happens during anesthesia, the equilibrium of the homeostasis is changed and this process is called stress. The animal's response to stressful stimuli is divided into three phases: behavioral changes, sympathetic system stimulation, and activation of the pituitary-adrenal hypothalamic axis.[33]

Activation of the pituitary-adrenal hypothalamic axis causes hormonal changes, including increase of growth hormone, cortisol, and stimulation of sympatho-adrenal efflux, and increased catecholamines, aldosterone glucagon, and changes in plasma proteins, blood glucose, sodium retention, and potassium depletion.[34] Increased sympathetic activity and noradrenaline levels also decrease insulin secretion and also increase hyperglycemia by increasing gluconeogenesis and decreasing glucose consumption.[35]

Ahmad *et al*. evaluated anesthetic stress after administration of dexmedetomidine and its combination with midazolam, fentanyl, and ketamine in dogs, and reported that plasma insulin levels were significantly reduced and plasma glucose levels increased at the end of the period. In their study, the level of cortisol decreased significantly.[36] The significant decrease in glucose and cortisol at 30 and 120 times in our study may suggest a better sedation effect of midazolam than methocarbamol. Note that adrenal cortical inhibition due to use of etomidate has limited its use in sedation and anesthesia.[37,38] It has been suggested that intravenous infusion of etomidate can inhibit adrenal cortical functions and cortisol levels decreases with time and continuous infusion of etomidate in dogs under general anesthesia.[39] As a matter of principle, it should be noted that stress-released glucocorticoid hormones can alter liver enzymes.[40] The same thing that happens when the animal is restrained before anesthesia. But, changes in liver enzymes attributed to the use of anesthetic drugs due to the production of toxic metabolites for the cells. For example, after the administration of ketamine-midazolam creatinine, urea, AST, and ALP were markedly increased.[33] Ketamine in combination with midazolam can affect the liver and midazolam is thought to be responsible for increased ALT, ALT, AST, and blood urea and creatinine activity.[41] Similarly, our study also showed significant changes in AST and ALT levels after administration of midazolam, methocarbamol, and etomidate. However, in a different study evaluating the effect of ketamine-midazolam on the clinical and hematological factors of dogs, it was found that only hematocrit showed a significant decrease and other blood parameters were unchanged. Also the status of biochemical parameters (urea, creatinine, GGT, ALP, ALT, AST, and LDH) was unchanged.[42] In other words, administration of different drugs to achieve sedation or anesthesia can lead to considerable or insignificant changes in liver enzyme levels.

In our study, no significant difference in AST activity was observed at the same time between the two evaluated groups but a notable increase in ALT activity was observed in MeE group compared to MiE group at times 5, 30, and 180 studies. However, no valid published article has been found to indicate a negative effect of methocarbamol on liver and hepatitis incidence in dog, as well as liver disease due to methocarbamol administration. In humans, the hepatic side effects of this drug have not been reported.[43] The results of the study showed that both the drug

regimens used in pre-anesthesia and anesthesia (etomidate-midazolam and etomidate-methocarbamol) caused minimal changes in the cardiovascular system of dogs. However, attention to hematobiochemical changes in the assayed groups revealed that administration of methocarbamol such as midazolam may be considered as part of the premedication compound, and further studies are warranted to be used in other studies.

Conflict of Interests

The authors declare no conflict of interest.

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