ORIGINAL ARTICLE

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

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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 as premedication.

Design- Experimental Study.

Animals- Fourteen native young female dogs

Procedure- Dogs were randomly allocated to 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) intravenously. In addition to recording anesthesia time table, physiological and hematobiochemical parameters were also measured at different time points in two groups.

Results- It was found that there was no significant difference between 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 time points. Significant increase in ALT and AST activities was observed in MeE group compared to MiE group at some time points.

Conclusion and Clinical Relevance- Intramuscular administration of methocarbamol or midazolam prior to etomidate not only has minimal cardiopulmonary effects and hematobiochemical changes in dogs but provide similar anesthesia and recovery time.

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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 systems. Etomidate is a short-acting anesthetic that may be prescribed for induction of anesthesia and sedation in some minor surgeries and therapeutic interventions including 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 lack of release of histamine after etomidate administration.[1] Etomidate 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 since it has minimal cardiovascular changes, even in hypovolemic dogs, it is an appropriate drug for induction and maintenance of anesthesia in total intravenous anesthesia (TIVA) technique. However, 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 dog. Therefore, premedication is recommended before induction of anesthesia with etomidate. Midazolam, 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 suppressing the central nervous system. The mechanism of its action is not well understood, however, 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 beside their useful properties may justify their beneficial use before administering etomidate to dogs. Therefore, in the current investigation, intramuscular administration of midazolam or methocarbamol before intravenous administration of etomidate was evaluated 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 number of 14 female indigenous dogs (5-7 months old) weighing 5.1-2.23 kg were included into the present study. Animals were transferred to the Animal House Center of Shahrekord University at least two weeks before the study and the their 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. They were starved 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) or 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 premedication, etomidate (2 mg/kg; Hypnomidate®, Piramal, UK) was injected intravenously, and physiological parameters such as RT, RR, and HR were recorded from time 0 (before midazolam or methocarbamol administration) and at 5 min intervals until complete recovery of the animals.

Clinical Evaluation

Following administration of etomidate, the time from administration and the loss of the pharyngeal reflex (the possibility of tracheal intubation) were recorded as induction time, and the time interval between the return of the pharyngeal reflex to emergence of the righting reflex (return of the animal to the external position) was recorded as recovery time. Therefore, the time between loss and return of the pharyngeal reflex was considered as duration of anesthesia (maintenance time).

Hematobiochemical Assessment

Blood samples were taken at times 0, 130, 180 minute, and 24 and 48 hours after drug administration to measure hematocrit (HCT) and cortisol hormone. For this purpose, the collected samples were sent to the laboratory using laboratory tubes with 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]

Statistical Analysis

The recorded data were 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 the parameters in the MeE group showed changes at various times, however, they were not significantly different from each other ($p > 0.05$). Whereas in the MiE group the HR parameter showed a significant increase in time point of 5 and again after its decrease at time points of 10 and 15, increased again at time point of 20 ($p < 0.05$). In other words, HR changes in this group were noticeably fluctuating, unlike the MeE group (Figure 1).
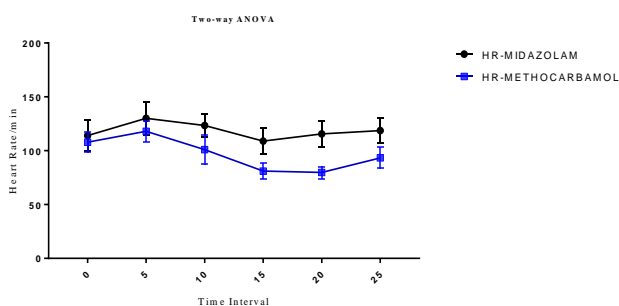
Figure 1. HR parameter changes during anesthesia in two groups of MiE and MeE

Comparison of the mean (± SEM) of RR between the two groups showed a significant increase in this parameter at time point 10 ($p < 0.05$). The intra-group analyses of the

parameter in the MiE group showed a significant increase at time ppoint of 5 and then considerable decrease and finally a significant increase at timepoint 20 ($p < 0.05$). However, in the MeE group there were no significant changes at different times (Figure 2).
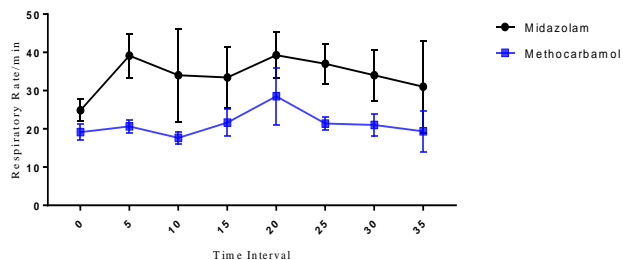
Figure 2. RR parameter changes during anesthesia in two groups of MiE and MeE

Comparison of the mean ($\pm$ SEM) of RT between the two analyzed groups showed a significant increase at time point of 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 time points. But, only at time point of 5 in the MiE group a significant increase was observed ($p < 0.05$).

Table 2 shows the mean ($\pm$ SEM) comparison of 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 showed no considerable difference at different time points ($p > 0.05$).

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

Comparison between groups showed a significant increase in the mean value of glucose parameter at time points of

120 and 180 in MeE group compared to MiE. In both groups, it was significantly increased at time points of 5. While there was no significant change in the MiE group, there was again a significant increase in mean values of glucose at 120 time point in MeE group ($p < 0.05$).

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

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

Significant increase in ALT activity at time point of 30 was observed in the MiE group. Then, at 120 and 180 minutes and 24 hours after anesthesia its activity was significantly decreased ($p < 0.05$). Considerable increase in the activity of the enzyme was observed in MeE group at 5 and 30 minutes after anesthesia. 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 ($\pm$ SEM) of AST in the MiE group only at time point of 30 and then recorded without any significant changes. However, in the MeE group, only time point of 24 showed a significant increase in the activity of AST (Table 2).

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

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 MiE and MeE groups.

Parameter	Group	Time						
		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 was returned from anesthesia (recovery time) in both groups tremor and muscle contraction were observed. Consistent with this study, Schwarzkopf *et al.* showed that anesthesia

with etomidate induced 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 was found that etomidate caused more rough recovery and more adverse reactions during anesthesia compared to propofol.[17]

There was no significant difference in duration of induction, maintenance, and recovery of anesthesia in the studied groups. In other words, methocarbamol and midazolam were 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, however, 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 administration of 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] The effects of etomidate as a suitable drug for cardiovascular stability has been described.[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 one is the reduction of the depth of anesthesia.[22] However, the simplest method for partial monitoring of the respiratory tract is to monitor 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 observed following midazolam administration. Consistent with our study, Castro et al. showed that the use of midazolam decreased minute and tidal volumes, however, the increased RR compensated the conditoin.[23]

The effect of etomidate on respiratory function is not fully known. Researchers reported 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, hypothermia, may occur in animals 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 body temperature was decreased following anesthesia due to CNS depression, 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 with its central effect can cause hypothermia.[28,29] However, the combination of a benzodiazepine with other anesthetics can produce variuos results,. 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 was not significantly changed after methocarbamol and midazolam administrations. In addition, no significant change was observed after etomidate administration. 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] Insignificant changes in red blood cells, leukocytes, hematocrit and hemoglobin were occured 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 is markedly decreased.[32] The results of the present study indicated a significant increase in glucose and cortisol after methocarbamol and midazolam administrations (time point 5). Basically, due to environmental stimuli like 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 in growth hormone, cortisol, stimulation of sympatho-adrenal efflux, increased catecholamines, aldosterone, glucagon, changes in plasma proteins, blood glucose, sodium retention, and potassium depletion.[34] Increased sympathetic activity and noradrenaline levels also decrease insulin secretion and also induce 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 was significantly decreased.[36] The significant decrease in glucose and cortisol at 30 and 120 time points in our study

could explain a better sedation effect of midazolam compared to 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] 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 are 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 remained unchanged. Also the status of biochemical parameters (urea, creatinine, GGT, ALP, ALT, AST, and LDH) were remained 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 time points of 5, 30, and 180 studies. However, no report was found to indicate a negative effect of methocarbamol on liver and hepatitis incidence in dog, as well as liver disease due to administration. In humans, the hepatic side effects of methocarbamol have not been reported.[43] The results of the

present 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 and midazolam may be considered as part of the premedication compound, and further studies are warranted to be conducted in other studies.

Conflict of Interests

The authors declare no conflict of interest.

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