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Effects of Midazolam or Midazolam-Fentanyl on Sedation and Analgesia Produced by Intramuscular Dexmedetomidine in Dogs

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ABSTRACT

The study was conducted to evaluate and compare sedation, analgesia and muscle relaxation, and other systemic changes produced by dexmedetomidine, dexmedetomidine-midazolam and dexmedetomidine-midazolam-fentanyl in 12 apparently healthy adult dogs divided into three groups (n = 4). In a prospective randomised blinded study, the dogs received 20 μg kg^{-1} dexmedetomidine (group A), 20 μg kg^{-1} dexmedetomidine and 0.2 mg kg^{-1} midazolam (group B), and 20 μg kg^{-1} dexmedetomidine, 0.2 mg kg^{-1} midazolam and 4 μg kg^{-1} fentanyl (group C), through intramuscular route. All the drugs were given simultaneously using separate syringes. Dexmedetomidine produced moderate sedation and muscle relaxation and mild to moderate analgesia with mild depression of laryngeal reflex. Addition of midazolam resulted in excellent muscle relaxation, deep sedation and moderate analgesia with moderate depression of laryngeal reflex. Weak time and down time were decreased whereas time to return of righting reflex and recovery time were increased. Heart rate, respiratory rate and rectal temperature and pulse oximeter values did not differ significantly between groups A and B. Addition of fentanyl further accentuated muscle relaxation, analgesia and produced deep sedation and allowed easy intubation without any further depression of clinical parameters. It reduced the onset time and increased recovery time further. It was concluded that addition of midazolam enhances the sedation and muscle relaxation produced by dexmedetomidine. The combination of fentanyl-dexmedetomidine-midazolam results in excellent analgesia, sedation and muscle relaxation with favourable conditions for intubation which may be used to perform diagnostic or minor surgical operations in dog.

Key words: Analgesia, midazolam, fentanyl, dexmedetomidine, anaesthesia

INTRODUCTION

Dexmedetomidine is an alpha-2 agonist with sedative, analgesic, muscle relaxant and anaesthetic reducing properties (Gertler *et al*., 2001). Dexmedetomidine produces dose dependent sedation and analgesia, however, sedation does not increase in proportion to the increase in dose but cardiorespiratory effects become more apparent (Kuusela, 2004). Thus, combinations of dexmedetomidine have been proposed with other sedatives and analgesics (Leppanen *et al*., 2006) and even local anaesthetics (Ahmed *et al*., 2008; Abosedira, 2008).

Midazolam, a water soluble imidazole benzodiazepine derivative is known to have minimal cardiorespiratory effects but is not a reliable sedative in dogs and cats and thus may be preferred in combination with opioids and alpha-2 agonists to induce sedation (Lemke, 2007). Midazolam has been combined with xylazine (Balicki *et al*., 2007), fentanyl (Natasa *et al*., 2007) and with medetomidine-butorphanol (Leonardi *et al*., 2007) for premedication in small animals.

Fentanyl is a synthetic μ-opioid receptor agonist, widely used for surgical analgesia and sedation (Huq, 2007). Being lipophilic in nature, it rapidly penetrates across biological membranes including the blood brain barrier which is responsible for its characteristic rapid onset and short duration of action (Hug and Murphy, 1979). Intramuscular or intravenous administration of fentanyl can cause bradycardia; however, it is not associated with a significant decrease in the mean arterial blood pressure (Grimm *et al*., 2005). Use of fentanyl along with midazolam for analgesia/anaesthesia in geriatric dogs has been associated with minimal modification of the vital parameters during surgical intervention (Natasa *et al*., 2007).

Though dexmedetomidine has been evaluated in dog alone and in combination with several other drugs viz butorphanol, diazepam, ketamine (Granholm *et al*., 2007; Selmi *et al*., 2003; Leppanen *et al*., 2006), no information is available on relative sedation/analgesia produced by dexmedetomidine alone and its combination with midazolam or dexmedetomidine-midazolam-fentanyl following simultaneous intramuscular administration in dogs.

The objective of the study was to evaluate the effects of midazolam or midazolam-fentanyl combination on the onset of sedation and analgesia and other systemic changes produced by dexmedetomidine in dogs.

MATERIALS AND METHODS

A prospective randomised blinded study was conducted on 12 client owned, mixed breed dogs of either sex, with a mean weight of 17.44±0.84 kg and mean age of 24.44±1.53 months. The animals were deemed healthy through physical examination. The study was conducted in the Division of Surgery and Referral Veterinary Polyclinic, Indian Veterinary Research Institute, Izatnagar, Bareilly, India from February to August 2010.

Design of work: The dogs were divided randomly into three equal groups, designated as group A, group B and group C. The base values of different parameters were recorded and then after a cooling time of 15 min the assigned drugs were administered in the three groups. In group A, 20 μg kg^{-1} dexmedetomidine (Dexdomitor; Orion Pharma, Finland) was administered intramuscularly. In the animals of group B, 20 μg kg^{-1} dexmedetomidine and 0.2 mg kg^{-1} midazolam (Mezolam; Neon Laboratories, Palghar, India) were administered. In group C, 20 μg kg^{-1} dexmedetomidine, 0.2 mg kg^{-1} midazolam and 4 μg kg^{-1} fentanyl (Fendrop; Sun Pharmaceutical India Ltd.) were used. All the drugs were given simultaneously by intramuscular route using separate syringes. The animals were left undisturbed for 10 min to allow the onset of effects. Recording of parameters for evaluation of sedation/analgesia began after 10 min and continued thereafter, at regular intervals up to 120 min.

Observations

Clinical observations: Relaxation of the jaw was taken as a measure of muscle relaxation during the study. It was evaluated by observing the resistance to opening of the jaws while pulling the

jaws apart. The status of jaw relaxation was recorded at 0, 10, 15, 20, 30, 45, 60, 75, 90, 105 and 120 min intervals on a 0 to 4 score scale as shown in the Table 1. At each interval mean value for jaw relaxation score was calculated and the muscle relaxation was graded as nil on a mean score of 0, very mild when the score was > 0 but < 1, mild when the score was = 1but < 2, moderate when the score was = 2 but < 3 and excellent when the score was 3.

Status of palpebral reflex was recorded as a measure of depth of sedation (Leppanen *et al*., 2006). It was assessed by observing a blink of the eyelids on touching the area around medial canthus of the eyes with index finger. The status of palpebral reflex was recorded at the same intervals as for the jaw relaxation on 0 to 3 score scale as shown in the Table 1. At each interval mean value for the palpebral reflex score was calculated and the sedation was graded as absent on a mean score of 0, mild when the score was > 0 but < 1, moderate when the score was = 1 but < 2, deep when the score was = 2 but < 3 and very deep when the score was 3.

Status of pedal reflex was recorded as a measure of depth of analgesia. It was assessed by observing the withdrawal reflex to the pinching of interdigital skin of a hind foot of the animal (Kuusela, 2004). The response of the animal was graded on a 0 to 3 score scale (Table 1) at the same interval as for the jaw relaxation. At each interval mean value for pedal reflex score was calculated and the analgesia was graded as no analgesia on a mean score of 0, very mild analgesia when the score was > 0 but < 1, mild analgesia when the score was = 1 but < 2, moderate analgesia when the score was = 2 but < 3 and complete analgesia when the score was 3.

Response to intubation was recorded to assess the status of laryngeal reflexes and feasibility of intubation during different stages of sedation/anaesthesia in all the animals. The response to intubation was recorded by attempting intubation at the same intervals as for the jaw relaxation. The response of the animals to intubation was recorded at the same intervals as for the jaw relaxation using a 0 to 4 score scale as given in Table 1. At each interval mean value for intubation score was calculated and the status of laryngeal reflex was graded as strong on a score of 0 to < 1, very mild depression when the score was = 1 but < 2, mild depression when the score was = 2 but < 3, moderate depression when the score was = 3 but < 4 and complete depression of laryngeal reflex when the score was 4.

Extent of salivation was recorded at different intervals as for the other reflexes and was graded from 0 to 3 using the score scale shown in Table 1. The person blinded to the treatment given to the animal allotted the score to the reflex responses.

Table 1: System of recording of various reflexes and responses (Adapted and modified after Amarpal *et al*. (1996)

	Score				
Parameter	0	1	2	3	4
Relaxation of jaw	Not allowing to open the jaws	Resistant to opening the jaws and closed quickly	Less resistance to opening the jaws and closed slowly	No resistance and jaws remain open	_
Palpebral reflex	Intact and strong (quick blink)	Intact but weak (slow response)	Very weak (very slow and occasional response)	Abolished (no response)	_
Pedal reflex	Intact and strong (strong withdrawal)	Intact but weak (animal responding slowly)	Intact but very light (slow and occasional response)	Abolished completely (no response)	_
Salivation	No salivation	Mild salivation	Moderate salivation	Excessive salivation	_
Response to intubation	Not permitting entry of tube in the mouth	Allowing entry but chewing	Allowing deeper entry but coughing intubation	Difficult intubation with coughing	Easy intubation without coughing

Weak time was recorded as the time elapsed from the time of injection of the drugs to the time of onset of incoordination / ataxia or drowsiness. Down time was recorded as the time that elapsed between the time of injection of the drugs and the time when the animal attained sternal recumbancy. The time to the return of righting reflex was recorded as the time elapsed from the injection of drug until the animal was able to regain sternal recumbancy. Standing recovery time was recorded as the time elapsed from the time of injection of the drugs until the animal attained standing position. Complete recovery was recorded as the time elapsed from injection of the drug (s) to the time when the animal stood and walked unassisted.

Other observation like urination, vomition and defaecation, if any, were also recorded.

Physiological observations: Heart Rate (HR), Respiratory Rate (RR), Rectal Temperature (RT) and oxygen saturation (SpO_2) were recorded before administration of the drug (s) at 0 min and at 10, 15, 20, 30, 45, 60, 75, 90, 105 and 120 min after administration of the drugs. Heart rate was recorded from pulse oximeter. Respiratory rate was measured by counting and recording the excursion of thoraco-abdomen. Rectal temperature was recorded with the help of a digital thermometer, the tip of which was placed deep into the rectum as per the standard procedure. Oxygen saturation (SpO_2) was measured by means of a pulse oximeter (Model 8600, pulse oximeter; Nonin Medical Inc. MPLS, MN). The probe of the instrument was applied to the toe web of the forelimb of the animal after clipping the hair around the site and cleaning with 70% alcohol (Huss *et al*., 1995).

Statistical analysis: The data were analysed for statistical significance using SPSS software version 15.0 (SPSS, Inc., Chicago, IL). One way analysis of variance and Duncan's Multiple Range Test (DMRT) were used to compare the means at different time intervals among different groups. Paired "t" test was used to compare the mean values at different intervals with their base values in each group. The subjective data generated from the scoring of various parameters were analysed using Kruskal Wallis test (Snedecor and Cochan, 1989). In each analysis, the differences were considered significant at a value of $p<0.05$.

RESULTS

Clinical observations: After 10 min of injection of the drugs, the mean score for jaw relaxation was 2.25 in group A, 2.5 in group B and 2.75 in group C. The score for jaw relaxation was suggestive of moderate muscle relaxation throughout the study period in group A and moderate to excellent in groups B and C. The score for jaw relaxation was significantly higher in groups B and C as compared to group A at 20 min interval. The score for jaw relaxation during the peak effect was highest in group C followed in decreasing order by groups B and A (Fig. 1).

The palpebral reflex score was suggestive of moderate sedation from 10 to 90 min in group A. The sedation was more in group B than that in group A and the animals of group B were deeply sedated between 10 and 60 min. The level of sedation in the animals of group C was almost similar to that in the animals of group B at most of the intervals. Comparison among the groups did not reveal any statistically significant difference in mean palpebral reflex score at different time intervals but the score was highest in the animals of group C followed by groups B and A (Fig. 2).

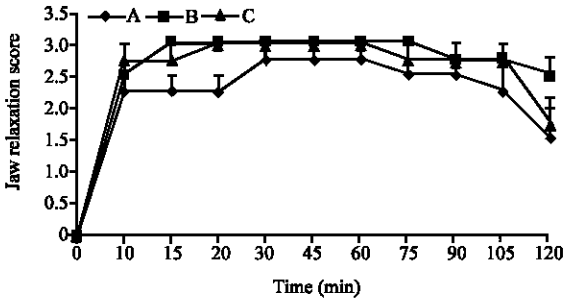

Fig. 1: Mean±SE jaw relaxation scores after administration of dexmedetomidine (group A), dexmedetomidine-midazolam (group B) and dexmedetomidine-midazolam-fentanyl (group C) (n = 4)

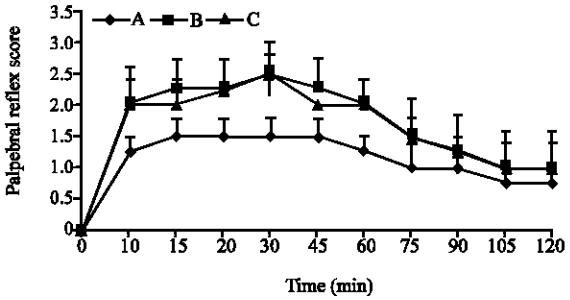

Fig. 2: Mean±SE palpebral reflex scores after administration of dexmedetomidine (group A), dexmedetomidine-midazolam (group B) and dexmedetomidine-midazolam-fentanyl (group C) (n = 4)

The pedal reflex score was suggestive of mild analgesia at 10 min after the administration of dexmedetomidine and moderate analgesia at 30 and 45 min in group A which was followed by mild analgesia during the rest of the study period. In group B, moderate analgesia was recorded from 30 to 60 min and mild to very mild analgesia during rest of the observation period. Analgesia in the animals of group C was relatively better than the animals of groups A and B. None of the animal in group A, one animal in group B and three animals in group C showed complete loss of pedal reflex for variable periods of time. The deepest analgesia was observed in animals of group C followed by the animals of groups B and A (Fig. 3).

In the animals of group A, laryngeal reflex showed very mild to mild depression and only 25% of the animals permitted intubation, that too with great difficulty and coughing. In the animals of group B, 50% of the animals permitted easy intubation and 25% of the animals with some difficulty. Mean score in group B were suggestive of moderate depression of laryngeal reflexes from 30 to 60 min. In group C, laryngeal reflexes were lost completely and a mean intubation score of 4 was achieved at 30 min interval which persisted up to 60 min interval. All the animals of this group permitted easy intubation for variable time periods. Comparison among the groups revealed significantly greater score in group C than that in groups A and B at 20, 30 and 45 min intervals. Intubation was maintained in the animals of group C for a mean time of 30 min (Fig. 4).

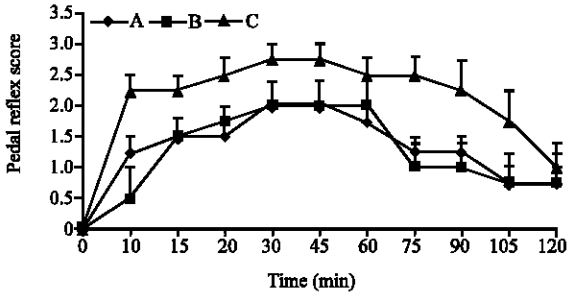

Fig. 3: Mean±SE pedal reflex scores after administration of dexmedetomidine (group A), dexmedetomidine-midazolam (group B) and dexmedetomidine-midazolam-fentanyl (group C) (n = 4)

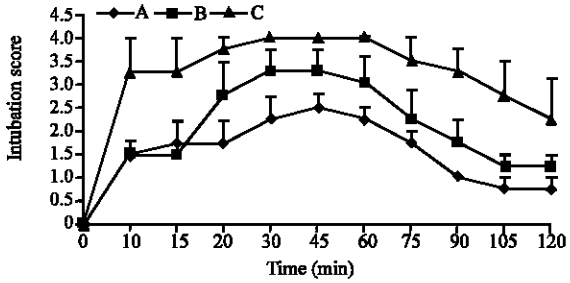

Fig. 4: Mean±SE response to intubation after administration of dexmedetomidine (group A), dexmedetomidine-midazolam (group B) and dexmedetomidine-midazolam-fentanyl (group C) (n = 4)

In groups A and B, no salivation was observed at any point of time during the observation period. In group C, salivation was seen at 10 min interval following the drug administration and continued up to 20 min. Two animals of group A and one animal of group C passed urine at the end of recording period. One animal of group A had vomition.

Weak time was shortest in the animals of group C (2.5±0.29 min), followed in increasing order, by group B (3.25±0.63 min) and group A (4.5±0.96 min). Mean weak time in group C was significantly different ($p<0.05$) from that of group A but it was not significantly ($p>0.05$) different from that of group B. Similarly, shortest down time was recorded in the animals of group C (5.25±1.03 min), followed, in increasing order, by that in group A (6.75±0.84 min) and group B (7.75±1.25 min) but the difference among the groups were not significant. Time to return of righting reflex in group A was 131.3±9.5 min, group B 128.8±8.14 min and in group C 140.5±9.24 min. Mean standing recovery time in group A was 144.3±9.01 min, group B 139±8.98 min and in group C 150±9.98 min. The differences in time to return of righting reflex and the mean standing recovery time were not significant among the groups (Fig. 5).

Complete recovery was recorded in 164.3±10.38 min in group A, 152.3±8.97 min in group B and 167.3±10.04 min in group C; however, the differences in the complete recovery times between the groups were not significant (Fig. 5).

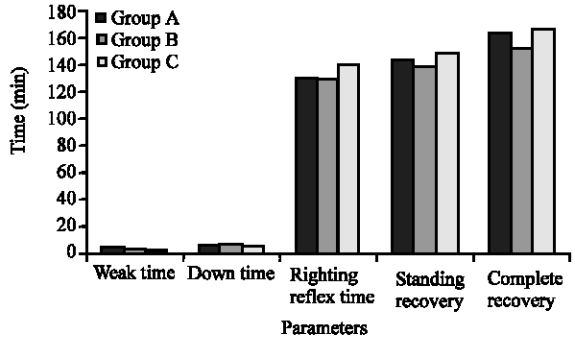

Fig. 5: Mean weak time, down time, righting reflex time, standing recovery time and complete recovery time (group A), dexmedetomidine-midazolam (group B) and dexmedetomidine-midazolam-fentanyl (group C) (n = 4)

Heart rate decreased gradually and significantly ($p<0.05$) up to 60 min after the injection of dexmedetomidine in the animals of group A, as compared to the baseline value. It remained significantly ($p<0.05$) lower than the baseline value until the end of the observation period (120 min). In group B, a significant ($p<0.05$) decrease in heart rate was recorded at 10 min following the drug administration. HR decreased further at subsequent intervals and remained significantly ($p<0.01$) lesser as compared to the base value. In group C, also a decrease in heart rate was recorded and it was significantly ($p<0.01$) below the baseline up to the end of the study (Table 2).

Respiratory rate decreased significantly ($p<0.05$) at 10 min after dexmedetomidine administration. The RR continued to be significantly ($p<0.05$) lower than the baseline up to 120 min of the study despite some improvement towards the end of the observation period. In group B, also a significant ($p<0.05$) decrease in RR was recorded at 10 min after the drug administration and RR continued to be significantly below the baseline throughout the period of the study. The decrease in respiratory rate was pronounced between 45 and 105 min where RR was significantly ($p<0.01$) lower than the baseline respiratory rate. Dexmedetomidine-midazolam-fentanyl combination caused only a slight and non-significant decrease in respiratory rate at 10 min. At subsequent intervals RR continued to decrease gradually up to 30 min, where the mean value was significantly ($p<0.05$) below the baseline. Thereafter, an improvement in RR was recorded and the values were only slightly below the baseline from 45 to 120 min of the study (Table 3).

The changes in the rectal temperature were not significant in any of the groups at most of the intervals except for group A where values of RT from 60 to 90 min were significantly ($p<0.05$) below the baseline values. In general an initial increase was followed by a decrease in RT in all the groups (Table 4).

Oxygen saturation did not show significant changes ($p>0.05$) at different point of time following administration of dexmedetomidine in group A, except at 30 min, where a significant increase in SpO_2 was recorded as compared to the baseline. SpO_2 remained decreased throughout the observation period in animals of groups B and C as compared to the base values but the decrease was significant only at 30, 45 and 90 min intervals (Table 5).

Table 2: Mean±SE values of heart rate in different groups at different time intervals

	Intervals (min)										
Number	0	10	15	20	30	45	60	75	90	105	120
A	106.80±14.19	42.75±2.05*[a]	37.75±3.75*	38.50±0.48*	38.00±1.78*	36.25±2.36*	36.50±2.47*	37.25±2.29*	44.50±5.90*	44.00±5.01*	43.50±4.17*
B	117.30±11.43	51.75±3.28*[a]	42.25±5.36**	42.00±5.03**	43.00±4.02**	41.75±4.10**	39.50±3.57**	44.75±3.07*	42.25±3.68**	38.25±3.92**	40.25±2.29*
C	115.80±6.61	39.50±0.87**[b]	37.75±1.44**	37.00±1.78**	34.75±2.87**	37.50±2.40**	36.25±1.65**	35.50±4.13**	38.75±1.80**	34.00±3.24**	41.75±6.81*

Values with different alphabets differ significantly ($p<0.05$) from each other in a column, *Differ significantly ($p<0.05$) from respective base values, **Differ significantly ($p<0.01$) from respective base values

Table 3: Mean±SE values of respiratory rate in different groups at different time intervals

	Intervals (min)										
Number	0	10	15	20	30	45	60	75	90	105	120
A	34.50±7.54	15.00±2.65*	13.25±0.85	13.00±1.35	14.00±1.78*	13.75±2.50*	13.75±3.00*	14.00±2.65*	14.00±3.37*	14.50±4.25*	19.75±8.75*
B	34.50±5.04	12.25±0.75*	11.25±0.63*	10.50±0.96*	10.25±0.85*	9.50±1.20*	9.50±1.20**	9.50±1.20*	10.25±0.95**	9.50±0.65*	10.00±1.08*
C	26.25±4.33	16.50±2.87	13.00±2.12	11.25±1.65	9.25±2.29*	10.50±1.71	11.25±2.06	11.00±1.87	11.50±1.85	10.75±2.14	10.75±2.14

*Differ significantly ($p<0.05$) from respective base values, **Differ significantly ($p<0.01$) from respective base values

Table 4: Mean±SE values of rectal temperature in different groups at different time intervals

	Intervals (min)										
Number	0	10	15	20	30	45	60	75	90	105	120
A	38.96±0.09	39.11±0.11	38.87±0.32	38.94±0.32	38.94±0.07	38.94±0.12[a]	38.71±0.03*[a]	38.13±0.24*	38.10±0.25*	38.00±0.31	37.85±0.34
B	38.87±0.15	38.91±0.12	38.85±0.2	38.79±0.19	38.64±0.20	38.42±0.07[b]	38.15±0.11[b]	37.93±0.06	37.63±0.14	37.54±0.17	37.28±0.14
C	38.71±0.32	39.08±0.14	38.95±0.16	39.10±0.12	38.84±0.21	38.63±0.10[b]	38.18±0.08[b]	37.92±0.12	37.42±0.32	37.51±0.27	37.25±0.26

Values with different alphabets differ significantly ($p<0.05$) from each other in a column, *Differ significantly ($p<0.05$) from respective base values

Table 5: Mean±SE values of oxygen saturation of haemoglobin (SpO_2) in different groups at different time intervals

	Intervals (min)										
Number	0	10	15	20	30	45	60	75	90	105	120
A	89.25±0.75[a]	91.50x±0.65*[b]	90.25±1.44	87.75±4.23	96.25±1.32*[a]	91.00±1.78	88.25±4.21	85.75±2.60	85.00±2.60	85.50±5.00	90.75±3.04
B	94.00±1.23[b]	84.75±3.10[b]	84.50±3.23	87.00±4.38	87.25±2.29*[b]	86.50±2.40*	91.25±1.50	82.75±2.56	79.00±2.45**	88.25±1.32	89.50±2.60
C	94.00±1.23[b]	89.50±1.76[ab]	88.50±2.53	87.25±4.37	87.50±2.22*[b]	86.75±2.32*	89.75±2.40	84.75±3.09	78.25±1.90**	88.25±1.32	87.50±2.60

Values with different alphabets differ significantly ($p<0.05$) from each other in a column, *Differ significantly ($p<0.05$) from respective base values, **Differ significantly ($p<0.01$) from respective base values

DISCUSSION

The study was conducted to evaluate and compare the effects of midazolam and midazolam-fentanyl on sedative, analgesic and anaesthetic effects of dexmedetomidine in dogs. The addition of midazolam or midazolam-fentanyl brought about clinically appreciable changes in sedation, analgesia and muscle relaxation produced by dexmedetomidine.

Moderate muscle relaxation observed in dexmedetomidine group may be attributed to inhibition of intra-neuronal transmission of impulses by the drug at the level of CNS (Gross and Branson, 2001). Status of muscle relaxation in the animals of group A corroborated the findings of Hanci *et al.* (2010) who reported good muscle relaxation after administration of dexmedetomidine in human patients. Greater muscle relaxation in group B could be attributed to the synergistic action of dexmedetomidine and midazolam, as reported by Bol *et al.* (2000). Midazolam, a benzodiazepine derivative is known to have good muscle relaxant action (Hellyer *et al.*, 1991; Ilkew *et al.*, 1998). Although, opioids by themselves do not induce muscle relaxation, however, their additive or synergistic interaction with benzodiazepine and/or alpha-2 agonist might have caused enhanced muscle relaxation in the animals of group C. The findings of the present study conformed to the observations of earlier researchers who reported greater muscle relaxation when dexmedetomidine or medetomidine was combined with opioids in dogs or cats (Ko *et al.*, 2000; Selmi *et al.*, 2003).

Only mild to moderate sedation in the animals of group A supported the observations of Huncke *et al.* (2010) who reported that dexmedetomidine was efficacious as a sole sedative in 50% subjects. The dose selected in the present study was moderate and further increase in the dose could have led to better sedation. Deep sedation in the animals of group B was achieved with the peak effect at 30 min interval which lasted up to 60 min. Hypnotic action of midazolam might have added to the sedation in the animals of group B. Midazolam induced hypnosis is related to modulation of GABA channel activity by occupation of benzodiazepine receptors, leading to GABA accumulation (Reves *et al.*, 1985). Midazolam as a single agent is reported to have a mild sedative effect in dogs, but it shows additive or synergistic activity when administered with other sedatives (Cwiek *et al.*, 2009; Karimpour *et al.*, 2005). In group C, deep sedation, similar to that in group B, was achieved. Absence of appreciable difference in the sedation of groups B and C could be attributable to less than additive interaction of midazolam and fentanyl and supported the observations of Schwieger *et al.* (1991). It has also been reported that assessment of sedation by evaluation of palpebral reflex may be less relevant in dogs not induced for general anaesthesia (Leppanen *et al.*, 2006) and minor differences might have not been recorded using the subjective assessment.

Dexmedetomidine alone produced mild to moderate analgesia. Analgesia produced by dexmedetomidine is mediated at the spinal level (Hayashi *et al.*, 1995) and by interruption of nociceptive pathways to the ventral root of dorsal horn which reduce spinal reflexes (Kending *et al.*, 1991; Savola *et al.*, 1991). The similar mechanisms might have been responsible for analgesia produced by dexmedetomidine in the present study. The depth of the analgesia did not differ between the groups A and B but longer duration of moderate analgesia was achieved in group B which could be attributable to the reported synergistic interaction of dexmedetomidine and midazolam (Bol *et al.*, 2000). Midazolam is reported to have a considerable effect on the nociceptive transmission in superficial dorsal horn (Kohno *et al.*, 2006) and cause pain relief (Akhlaghi and Rajaei, 2008). The analgesia achieved in group C was deeper and of longer duration than that of groups A and B. The greater analgesia in group C could be attributed to the action of

μ opioid agonist, fentanyl. It is 80 to 100 times more potent than morphine (Thurmon *et al.*, 1999) and has synergistic interaction with alpha-2 agonists (Salmenpera *et al.*, 1994).

Very mild to mild depression of laryngeal reflex was achieved when dexmedetomidine alone was used because of its hypnotic action. Depression of laryngeal reflex was more in group B as compared to group A. This increase in mean intubation score may be explained on the basis of reported synergism between midazolam and dexmedetomidine (Bol *et al.*, 2000). Easy intubation was achieved in group C, at 30 min after the drug administration which lasted up to 60 min. The loss of the reflex corresponded to the peak effect achieved by fentanyl at 30 min following intramuscular administration (Thurmon *et al.*, 1999). A synergistic interaction between dexmedetomidine, midazolam and/or fentanyl could be responsible for abolition of the reflex and better conditions for intubation in the animals of group C (Ben-Shlomo *et al.*, 1990; Salmenpera *et al.*, 1994).

Dexmedetomidine causes decrease in salivation, other secretions and bowel motility (Gertler *et al.*, 2001) which might be responsible for the absence of salivation in group A. Although, midazolam has been reported to cause drooling of saliva (Court and Greenblatt, 1992; Butola and Singh, 2007), absence of salivation in the animals of group B might be due to more potent action of dexmedetomidine. In the animals of group C, mild salivation was recorded which might be due to the action of fentanyl or complete depression of reflexes and the inability of the animals to swallow saliva produced in small quantities.

Weak time in the animals of group A was almost similar to that reported by Amarpal *et al.* (1996) after the administration of medetomidine in dog. The rapid onset of effects of medetomidine has been attributed to its lipophilic property (Amarpal *et al.*, 1996; Singh *et al.*, 2005). Dexmedetomidine, an isomer of medetomidine was also thought to act in a similar way as medetomidine. The decrease in the weak time in the animals of group B and group C as compared to that in group A may be attributable to the synergistic action of midazolam and fentanyl with dexmedetomidine as reported by Ben-Shlomo *et al.* (1990) and Salmenpera *et al.* (1994). A synergist interaction may be expected when the drugs acting on different site are administered simultaneously. A reduced weak time has been reported by Amarpal *et al.* (1996), when pentazocine was administered with medetomidine as compared to medetomidine alone. Down time represents the stage of the onset of effects of the drugs when animals become adequately depressed to be unable to support weight. Due to rapid onset of effects of dexmedetomidine the down time was recorded in 6.75±0.84 min in the animals of group A which was similar to that of medetomidine as reported by Pratap *et al.* (1997). A further decrease in the down time in the animals of groups B and C could be attributable to the simultaneous actions of these drugs at different sites in the CNS leading to faster onset of CNS depression.

Prolonged time to return of righting reflex in group A supported the observations of Kuusela *et al.* (2000) who reported that dogs administered with dexmedetomidine intravenously at the dose rate of 20 μg kg^{-1}, were laterally recumbent at least up to 90 min of the observation period. Return of righting reflex was achieved a bit earlier by the animals of group B as compared to that of group A. The results were in accordance with the observations of Cwiek *et al.* (2009) who found that dogs premedicated with midazolam-xylazine needed a shorter time to regain consciousness and motor functions compared to those premedicated with xylazine alone. Increased time to return of righting reflex in group C probably resulted from the pronounced synergistic action among dexmedetomidine, midazolam and fentanyl resulting in deeper sedation and

reduced metabolic activity to delay redistribution and metabolism of the drugs in this group (Ko *et al*., 2000). Standing recovery time and complete recovery time increased with increase in number of the drugs (groups B and C) used which could be correlated with increased sedation and decreasing metabolic rate in these groups (Ko *et al*., 2000) owing to synergistic interaction between the drugs.

Vomition was not a problem in the present study. The vomition occurred only in 1 animal of group A which could be due to alpha-2 adrenoceptors mediated emetic action. The alpha adrenoceptor-mediated emesis does not involve beta adrenergic, cholinergic, dopaminergic, histaminergic, serotonergic and opioid receptors in the emetic pathway (Hikasa *et al*., 1992). None of the animals of groups B and C had vomition which conformed to the observations of Cwiek *et al*. (2009) who recorded 50% reduction in vomition rate when midazolam was used with xylazine compared to xylazine alone.

Initial decrease in the HR may be attributable to alpha-2 agonist mediated vasoconstriction leading to reflex bradycardia (Lemke, 2004). Dexmedetomidine causes profound bradycardia but preserves blood pressure when administered intravenously (Kuusela *et al*., 2001). Results of the present study are in conformity with the earlier studies in dogs administered with dexmedetomidine intramuscularly 10 μg kg^{-1} where bradycardia was evident during 5 h of monitoring (Kuusela, 2004). Decrease in the HR in the animals of group B could be mainly due to the effects of dexmedetomidine which has been reported to cause greater reduction in heart rate as compared to midazolam (Chang *et al*., 2009). Pronounced decrease in heart rate in group C may be attributable to the action of fentanyl and its synergistic interaction with dexmedetomidine and midazolam which might increase the degree of bradycardia induced by dexmedetomidine (Salmenpera *et al*., 1994).

Dexmedetomidine results in a dose dependent depression in respiratory rate and slope of CO_2 response curve (Sabbe *et al*., 1994). Administration of medetomidine or dexmedetomidine has been found to decrease respiratory rate (Amarpal *et al*., 1996) with minimal effects on blood gases in dogs (Kuusela *et al*., 2000). Decreased respiratory rate in group A, conformed to the observation of these authors. In group B, decrease in respiratory rate was more as compared to the animals of group A which might be attributed to midazolam administration (Butola and Singh, 2007). In a study, midazolam was found to produce more respiratory depression as compared to dexmedetomidine in rabbits (Chang *et al*., 2009). Lesser decrease in respiratory rate in group C might signify the compensatory mechanism to counter the hypoxic effects of bradycardia and possible cardiovascular depression in the animals of group C.

Decrease in rectal temperature due to dexmedetomidine could be attributed to the activation of alpha-2C receptors which mediate hypothermia (Lemke, 2004). In the animals of group A, decrease in rectal temperature after the onset of effects might also be attributed to a decrease in heat production due to decreased muscular activity and to direct effect of the drugs on hypothalamus (Virtanen, 1989). A gradual decrease in rectal temperature following intravenous dexmedetomidine administration was observed by Raekallio *et al*. (2005); however, this decrease was non- significant throughout the study period of 90 min. In animals of groups B and C reduced muscular activity and sedation due to dexmedetomidine- midazolam or dexmedetomidine-midazolam-fentanyl induced deep sedation might have led to decrease in rectal temperature.

Low pulse oximeter readings were indicative of reduced arterial oxygenation and diminished tissue perfusion. However, possible vasoconstriction might have also led to low pulse oximeter readings (Leppanen *et al*., 2006). Initial decrease in SpO_2 in the animals of all the groups may be

attributable to vasoconstriction caused by dexmedetomidine as reported by Kuusela *et al.* (2000). Low arterial oxygen concentration could also be caused by respiratory depression due to sedation or even due to technical error (Leppanen *et al.*, 2006). The decrease in SpO_2 in the present study was only occasional and transient and SpO_2 values were fairly maintained throughout the most of the observation period.

CONCLUSION

It was concluded that addition of midazolam increased the sedation but not analgesia produced by dexmedetomidine and most of the animals could not be intubated. The co-administration of midazolam and fentanyl with dexmedetomidine produced excellent analgesia and sedation and favourable conditions for intubation. The combination may be used for deep sedation and analgesia to perform diagnostic or minor surgical operations in dog.

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