STUDIES ON THE EFFECT OF MEDETOMIDINE VERSUS ROMIFIDINE IN BUFFALO CALVES
(With one Table and 12 Figures)

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SUMMARY

Five healthy buffalo calves were used in this study in a crossover design with an interval of three weeks. In group I, the five calves received...
medetomidine 10 µg/kg intravenously while in group II, the same five calves, after three weeks, were injected i.v. with romifidine 50 µg/kg. The calves were evaluated at baseline and then at 5, 15, 30, 45, 60, 75 and 90 minutes after drug administration for respiratory rate, heart rate, degree of sedation, muscle relaxation, and response to pedal and pinprick reflexes. Weak time, down time and recovery time were recorded in both groups. ECGs were recorded in all animals at baseline, 5, 10, 15, 30, 45 and 60 min post-injection. Blood samples were taken at baseline, 5, 15, 30, 60 and 90 minutes after drug administration for determination of serum glucose, urea, AST, LDH and CPK. Heart rate decreased significantly in both groups. ECG findings in group I revealed increase in the R-R interval while in group II there was an atrioventricular (AV) heart block. Moderate sedation, mild muscle relaxation and mild analgesia were recorded in both groups. There was significant increase in serum glucose in both groups, while group II only showed significant increase in serum urea. Serum AST, LDH and CPK increased significantly in both groups compared to baseline values. It was concluded that medetomidine and romifidine are considered beneficial for safe investigation and clinical examination in buffalo calves but further restraint or conjunction with other local or general anesthetic is needed for surgical approaches. However, medetomidine is considered superior to romifidine because the later induces prolonged cardiopulmonary depression and comparatively higher serum levels of urea and cardiac enzymes.

Key words: Medetomidien, romifidine, buffalo calves

INTRODUCTION

The α2-adrenoceptor agonists (α2-agonists) are popular in veterinary and human medicine for use as anxiolytics, analgesics, and pre-anaesthetic sedatives. Moreover, in veterinary practice, the relatively new α2-agonist, (4-[1-(2, 3-dimethylphenyl) ethyl]-1H-imidazole hydrochloride) (Medetomidine), is widely used for sedation and to reduce the general anesthetic requirement (Kastner, 2006). Medetomidine is the most selective α2-agonist used in veterinary medicine and produces sedation and analgesia in dogs (Malm et al., 2007), in goats (Kinjavdekar et al., 2007), in cats (Westropp et al., 2007), and in horses (Umar et al., 2007).
Romifidine is an imino-imidazolidine derivative, selective and a
new $\alpha_2$-adrenoceptor agonist drug that is mostly administered
systemically to bring about sedation and analgesia in sheep (Celly et al.,
1997 and Kastner, 2006), dogs (Gomez-Villamandos et al., 2005),
horses (Figueiredo et al., 2005), and spinally in goats (Kinjavdeka et
al., 2006; and Kinjavdekar et al., 2007). However, no studies were
conducted to compare the effects of the IV injection of medetomidine
and romifidine in buffaloes.

Therefore, the objective of this study is to assess and compare the
analgesic, sedative, and cardiopulmonary effects of intravenous
injection of medetomidine versus romifidine in buffalo calves.

MATERIALS AND METHODS

Animals

Five healthy buffalo calves of both sex (three males and two females),
12- 18 months of age weighing 110-150 kg were used in this study in a
crossover design with an interval of three weeks. The buffalo calves were
kept in a controlled environment and maintained under uniform managerial
practice for feeding, watering and housing at the educational farm of the
Faculty of Veterinary Medicine, Benha University. The food was withheld
for at least 24 hours prior to experimentation. The animals were restrained
with thick cotton ropes during the experiment.

Drugs

The dosages of medetomidine and romifidine were selected on the
basis of a pilot study conducted on three buffalo calves guided with doses
cited in the previous literatures. The drugs and their dosages used in this
study were as follows. In group I, the five calves received medetomidine
(Domitor®, 1 mg/ml, Pfizer Pharma GmbH) 10 µg/kg intravenously (i.v.).
In group II, the five calves, after three weeks, were injected i.v. with
romifidine (Sedivet 10 mg/ml, Boehringer Ingelheim, Burlington, ON,
Canada) 50 µg/kg.

Evaluation of Sedation

The sedation induced by medetomidine (group 1) was compared
with that induced by romifidine (group II) using five buffalo calves in
each group. Each animal was injected i.v. with the two agents with three
weeks interval in a crossover design. After recording the baseline values
(pre-induction) of respiratory rate, and heart rate as well as ECG
tracing using an ECG monitor (Fukuda Denshi Co., Ltd. Tokyo, Japan) and collecting blood samples, medetomidine and romifidine were administered to animals of group I and Group II, respectively. The buffalo calves were evaluated at time 0 (pre-induction) and then at 5, 15, 30, 45, 60, 75 and 90 minutes after drug administration for respiratory rate, and heart rate, as well as, degree of sedation, muscle relaxation, and response to pedal and pinprick reflexes. Scores awarded to the later four parameters were according to Pawde, et al. (2000) as follows: sedation (score 0–3): 0, no sedation (standing alert, keeping the head high, eyes open); 1, mild (standing tired, lowering of head, drooping of eye lids); 2, moderate (recumbent but able to sit without support); 3, strong (unable to sit without support); muscle relaxation (score 0–3): 0, absent (tightly closed jaws and stiff limbs); 1, mild (mild resistance to opening of jaws and bending of limbs); 2, moderate (moderate resistance to opening of jaws and bending of limbs); 3, complete (no resistance to opening of jaws, bending of limbs and flaccid abdomen); pedal reflex (score 0–3): 0, intact; 1, weak; 2, very weak; 3, abolished; response to pinprick (score 0–3): 0, strong; 1, weak; 2, very weak; 3, abolished.

Muscle relaxation was observed in the muscles of abdomen, legs and jaws. The ease with which the jaws of recumbent animals could be opened and their hind limbs could be bent without resistance, as well as the flaccid abdomen could be pressed was recorded as the extent of muscle relaxation (Sharma et al., 2004).

We measured another three important sedation indicators in this study. The weak time was the time (minutes) elapsed from the time of injection of the drug to the time when the animal showed ptosis (dropping) of the head. Down time was the time from the administration of medetomidine or romifidine to sternal/lateral recumbency. Recovery time was recorded in both groups as the time elapsed between the time of administration of drugs and the time when the animal was able to walk unassisted.

Electrocardiography

ECG traces were recorded in all animals of both groups using lead II and ECG Monitor (Fukuda Denshi Co., Ltd. Tokyo, Japan) at 0 (pre-injection), 5, 10, 15, 30, 45 and 60 minutes post-injection according to Ghanem (1997). Briefly, the right forelimb electrode (RA) and the left forelimb electrode (LA) were attached to the right and left elbow joints, respectively through needles inserted subcutaneously in these areas. Both hind limb electrodes (RL and LL) were attached to the right and left stifle joints, respectively through needles inserted subcutaneously in these areas (Fig.1). The R-R interval was calculated as an indicator of the cardiac frequency (Huber et al., 2004). The monitor speed was set at 25 mm/sec and heart rate was recorded in all traces.
Serum Analysis

Blood samples were collected from jugular vein at 0 (pre-induction) and then at 5, 15, 30, 60 and 90 minutes after drug administration and serum was separated by centrifugation for determination of serum glucose, urea, AST, LDH and CPK.

Statistical Analysis

The changes of heart rate and respiratory rate, as well as, serum biochemical values were compared with their baseline values using one way ANOVA. In addition, weak time, down time and recovery time in the two groups were also compared using one way ANOVA and means were compared using Duncan's multiple range test. A subjective scoring system of sedation, muscle relaxation and pedal and pinprick reflexes was used and data from calves of group I and II were compared by Wilcoxon's signed ranks test (P < 0.05 considered significant). The statistical analyses were all performed using SPSS (version 13 for windows; SPSS Inc., Chicago, IL. USA).

RESULTS

Changes in Heart Rate

Heart rate decreased significantly in both groups (Fig. 2). The fall in heart rate in group II was greater than that of group I as compared to the baseline values. In group I, the fall in heart rate was significant up to 75 minutes after injection of medetomidine, while at 90 minutes after
its injection, the fall in heart rate became non significant from the baseline value. On the other hand, the fall in heart rate was significant all over the study period in group II.

**ECG Findings**

ECG findings (Fig. 3) of medetomidine-injected buffalo calves showed a marked reduction in the heart rate (bradycardia) denoted by prolongation of the R-R interval (the period between 2 successive R waves) (Fig. 4). The myocardial contraction increased markedly as denoted by the increase in the amplitude of QRS complex starting at 5 minutes post-injection. The normal biphasic (bifid) P wave became monophasic. Moreover, the T waves became monophasic instead of biphasic and had higher amplitude. The ventricles began to return to their normal contraction, heart rate began to return to its baseline value and the biphasic P wave returned to its normal figure 60 minutes after injection.

ECG findings of romifidine-injected buffalo calves showed bradycardia accompanied by second degree of atrioventricular (AV) heart block that appeared after 5 minutes of injection and continued until the end of experiment (60 minutes). The AV heart block appeared as that some P waves were not followed by QRS or T waveforms. This AV heart block indicated that some impulses did not transmit from atria to ventricles (Fig. 3).
Fig. 3: Lead II ECG tracing of a buffalo calf from group I, injected with medetomidine (left column), and a buffalo calf from group II, injected with romifidine (right column). The tracings were recorded before (baseline) and after injection in different time series. (speed: 25mm/sec). Notice the AV heart block as P waves (arrows) are not followed by QRS-T waves. HR is the heart rate.

Fig. 4: R-R intervals (in milliseconds, ms) in ECG tracings recorded from buffalo calves injected with medetomidine (group I) and romifidine (group II).
Changes in Respiratory Rate

Respiratory rate (Fig. 5) decreased significantly at 15, 30 and 45 minutes after medetomidine injection in group I, while it decreased significantly at 5, 15, 30, 45, 60 and 75 minutes after romifidine injection in group II.

![Graph showing changes in respiratory rate](image)

Fig. 5. Respiratory rates (Mean ± SD) in buffalo calves injected with medetomidine (group I) and romifidine (group II)

Changes in Weak, Down and Recovery Times

In group I, the mean weak time (5.8 minutes) and down time (9 minutes) were non-significantly higher than the weak time (3 minutes) and down time (6.4 minutes) in group II, respectively. However, recovery time (108 minutes), after romifidine sedation, was significantly longer than that after medetomidine sedation (71 minutes) (Fig. 6). All animals of both groups took the same sternal recumbent position resembles to the milk fever position with head and neck deviated laterally.

![Graph showing weak, down, and recovery times](image)

Fig. 6: Weak time, down time and recovery time (Mean ± SD) in buffalo calves injected with medetomidine (group I) and romifidine (group II).
Changes in sedation, muscle relaxation, pedal and pinprick reflex

There was no significant difference in the degree of sedation (Table 1) between the two groups allover the study period (P > 0.05). Moderate sedation was recorded in all except for one animal after injection of medetomidine and romifidine. During moderate sedation, the animals showed sternal recumbency and took a position resemble to the milk fever position allover the period from 15 - 60 minutes after injection (Fig. 7A and B). Thereafter, a mild sedation followed for another 15 minutes during which the calves were standing tired and their heads were lowered (Fig. 7C and D). Only one animal from five administered with medetomidine showed a strong degree of sedation than other animals. This calf was laterally recumbent and unable to sit without support for 60 minutes after medetomidine injection; thereafter it began to sit on sternal recumbent position for another 20 minutes.

Although the degree of sedation was moderate in the two groups without significant difference, romifidine induced longer sedation than did medetomidine. Excessive salivation was observed in all animals allover the period of sedation.

Table 1: Difference between romifidine and medetomidine (Wilcoxon's signed ranks tests) in degree of sedation, muscle relaxation, pedal and pinprick reflexes (mean ranks and P-value).

<table>
<thead>
<tr>
<th>Time</th>
<th>Degree of sedation</th>
<th>Muscle relaxation</th>
<th>Pedal reflex</th>
<th>Pinprick reflex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean rank</td>
<td>P-value</td>
<td>Mean rank</td>
<td>P-value</td>
</tr>
<tr>
<td>Baseline</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 min.</td>
<td>1.5</td>
<td>0.414</td>
<td>0.317</td>
<td>0.083</td>
</tr>
<tr>
<td>15 min.</td>
<td>2</td>
<td>0.083</td>
<td>0.317</td>
<td>0.317</td>
</tr>
<tr>
<td>30 min.</td>
<td>1</td>
<td>0.317</td>
<td>0.564</td>
<td>0.317</td>
</tr>
<tr>
<td>45 min.</td>
<td>1</td>
<td>0.317</td>
<td>0.655</td>
<td>0.317</td>
</tr>
<tr>
<td>60 min.</td>
<td>1.5</td>
<td>1.0</td>
<td>0.317</td>
<td>0.317</td>
</tr>
<tr>
<td>75 min.</td>
<td>2.5</td>
<td>0.317</td>
<td>1.0</td>
<td>0.0</td>
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<tr>
<td>90 min.</td>
<td>0</td>
<td>1.0</td>
<td>1.0</td>
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</table>
Fig. 7: Degrees of sedation after injection of medetomidine (A&C) and romifidine (B&D). Moderate sedation with sternal recumbency and milk fever position is seen in A&B (25 minutes post injection) while mild sedation is seen in C&D and the animals stand tired. Excessive salivation is observed (60 minutes post injection).

There was no significant difference in the degree of muscle relaxation (Table, 1) between the two groups (P>0.05). Mild degree of muscle relaxation (mild resistance to opening jaws and bending of limbs but with dropped head) was recorded in both groups during the period from 5-60 minutes after injection of medetomidine and romifidine. Thereafter, the calves maintained their muscle tone fully.

The changes in pedal and pinprick reflexes as indicators for the degree of analgesia (Table, 1) were not significantly different in the two groups. Only mild depression of both reflexes was recorded allover the period from 5-60 minutes after injection of medetomidine and romifidine.

**Change in Serum Glucose, Urea, AST, LDH and CPK**

Although there was a significant increase in serum glucose (Fig. 8) in both groups as compared to the baseline values, this increase was higher in group II after injection of romifidine than that in group I after injection of medetomidine. Group II showed a significant increase in serum urea (Fig. 9) level at 15, 30, 60 and 90 minutes after romifidine injection while group I showed mild non significant increase. Serum AST (Fig. 10) and LDH (Fig. 11) increased significantly in both groups all over the study period. CPK (Fig. 12) increased significantly in group I at 30, 60 and 90 minutes after medetomidine injection while greater significant increase was shown in group II all over the study period.
Fig. 8. Serum glucose (mean ± SD) in buffalo calves injected with medetomidine (group I) and romifidine (group II)

Fig. 9. Serum urea (mean ± SD) in buffalo calves injected with medetomidine (group I) and romifidine (group II)

Fig. 10. Serum AST (mean ± SD) in buffalo calves injected with medetomidine (group I) and romifidine (group II)
DISCUSSION

Alpha2-adrenoceptor agonists are used to produce sedation, analgesia, and muscle relaxation in sheep (Celly et al., 1997), horses (Freeman et al., 2002; and Figueiredo, et al., 2005) and goats (Kinjavdekar, et al., 2007).

The alpha-2 agonists have marked effects on the cardiovascular system. These effects include bradycardia, an initial hypertension followed by hypotension, a decrease in cardiac output, and an increase in systemic vascular resistance (Freeman et al., 2002). In this study, heart rate decreased significantly after injection of both
medetomidine and romifidine in buffalo calves. The fall in heart rate after romifidine injection was greater than that after medetomidine injection as compared to the baseline values. Similarly, romifidine induced bradycardia, and decreased cardiac output in horses (Figueiredo et al., 2005). Our result was also consistent with the findings of Singh et al., (2005) who found that injection of medetomidine alone markedly reduced heart rate compared to medetomidine and ketamine in buffaloes. Westropp et al. (2007) also stated that heart rate decreased after medetomidine administration in healthy cats. The significant fall in heart rate, in this study, continued up to 75 minutes after injection of medetomidine, while it continued allover the study period after romifidine injection. This result was consistent with that of Freeman et al. (2002) who demonstrated that the cardiovascular effects of romifidine in horse are prolonged. Moreover, Kinjavdekar et al. (2006) stated that the heart rate decreased significantly soon after the administration of romifidine subarachnoidally in goats until the end of the observation period.

ECG findings, in this study, showed that medetomidine injection induced marked bradycardia in buffalo calves indicated by prolongation of the R-R interval. This could be attributed to decreased sympathetic outflow from the central nervous system and increased vagal tone (Bueno et al., 1999). Similarly, the ECG changes in goats administered with medetomidine (0.01 mg/kg body wt) in the subarachnoid space produced bradycardia and prolongation of R-R interval and Q-T intervals (Kinjavdekar et al., 1999). They added that the increased amplitude/duration of QRS complex indicates increased ventricular depolarization while increased amplitude of T wave may indicate myocardial hypoxia. The P wave amplitude, in this study, did not change by medetomidine injection, which indicated that the atrial depolarization was not affected after the injection, a result that coincided with Singh et al., (2005). The heart rate began to return to its baseline value 60 minutes post medetomidine injection and both P and T wave became biphasic and had returned to normal amplitudes. These changes suggested that the cardiac changes of medetomidine in buffalo calves continue for 60 minutes after injection and had returned to baseline afterwards.
The ECG findings, in this work, showed normal sinus rhythm before injection of romifidine. However, romifidine injection produced bradycardia associated with second degree atrioventricular (AV) heart block. Similarly, romifidine was demonstrated to induce marked bradycardia and AV heart block in horses that was obliterated by administration of atropine sulphate, 5 minutes before injection (Gasthuys et al., 1990). Our results were also similar to those reported earlier following subarachnoid injection of romifidine in goats in which the important ECG changes recorded were bradycardia, increased PR and QT intervals and increased amplitude of the T waves (Kinjavdekar et al., 2006). The central sympatholytic effect of romifidine may be responsible for the atrioventricular heart block (Wagner et al., 1991) while the bradycardia could be attributed to inhibition of sympathetic tone from CNS and vagal stimulation (Ruffolo et al., 1993). Figueiredo et al., (2005) added that second AV block occurred following the administration of romifidine and the incidence of arrhythmias declined as heart rate increased and signs of sedation decreased.

Alpha2-adrenoceptor agonists decrease respiratory rate and heart rate. Hypoventilation usually does not occur because the depth of breathing increases to maintain minute ventilation (Yamashita et al., 2002). In this study, respiratory rate decreased significantly only at 15, 30 and 45 minutes after medetomidine injection in group I, while it decreased significantly at 5, 15, 30, 45, 60 and 75 minutes after romifidine injection in group II. This result suggests that romifidine has extended respiratory suppression than medetomidine. Similarly, romifidine caused reduced respiratory rate in horses (Figueiredo et al., 2005). Therefore, it was recommended that rapid IV injection of alpha2 agonists without supplementary oxygen should be avoided whenever hypoxemia may be critical (Kastner, 2006). Yamashita et al. (2007) suggested also that medetomidine/ ketamine/ midazolam combination for total intravenous anesthesia in horses has a considerable promise as an injectable technique that can be used to produce extended anesthesia under field conditions. However, they added that inspired air should be supplemented with oxygen to prevent hypoxemia.
Moderate sedation was recorded in the animals of this study after injection of medetomidine and romifidine. These animals showed sternal recumbency and took a position resemble to the milk fever position allover the period from 15 - 60 minutes after injection. Thereafter, mild sedation was observed for another 15 minutes and the calves were standing tired and their heads were lowered. Similarly, Robertsona and Taylor, (2004) stated that alpha2 agonists, primarily medetomidine, dexmedetomidine and originally xylazine are commonly used in cats for their sedative and anesthetic properties. Our result was also in agreement with Figueiredo et al. (2005) who stated that romifidine is a potent and selective alpha2 adrenoceptor agonist that produces sedation, muscle relaxation, reluctance to move, reduced responsiveness to environmental stimuli. The results agreed also with Yamashita et al. (2002) and Yamashita et al. (2007) who reported that medetomidine has a high α2 adrenoceptor selectivity and produces sedative and analgesic effects at small doses in horses. In this study, the sedative effect (weak time) started 5.8 minutes and 3 minutes after medetomidine and romifidine injection, respectively. However, the calves began to recover from sedation 108 minutes after romifidine sedation the time which was significantly longer than that after medetomidine sedation (71 minutes). Although the degree of sedation was moderate in the two groups without significant difference, romifidine induced longer slightly deeper sedation than did medetomidine based on the signs of sedation and responsiveness of the sedated calves to their environment. Only one animal from five administered with medetomidine, in this study, showed a deeper degree of sedation than other animals. This calf was laterally recumbent and unable to sit without support for 60 minutes after medetomidine injection; thereafter it began to sit on sternal recumbent position for another 20 minutes.

Mild degree of muscle relaxation (moderate resistance to opening of jaws and bending of limbs but with dropped head) was recorded in both groups of our study during the period from 5-60 minutes after injection of medetomidine and romifidine without significant difference. Thereafter, the calves maintained their muscle tone fully. Nevertheless, Freeman and England (2000) and Figueiredo et al. (2005) reported that alpha2-adrenoceptor agonists produce excellent muscle relaxation of the muscles of the head, neck, and ears in horses followed by drooping of the head, ears, and lips. They explained that these effects are centrally-mediated, are well - correlated with the degree of sedation, and have become
widely accepted as objective methods for the assessment of the depth and duration of the sedation provided by alpha2-adrenoreceptor agonists. These authors did not practice any attempts to open the animal’s jaws or bending limbs for assessment of degree of relaxation as we did in our study and although there was drooping in the head and neck, the degree of relaxation was expressed as mild according to the assessed parameters. Muscle relaxation was also reported in the study of Robertona and Taylor (2004) in cats.

Analgesia is an important quality of alpha2 adrenoceptor agonists (England and Clarke, 1996). Only mild analgesia as expressed by mild depression in both pedal and pinprick reflexes was recorded during the period from 5-60 minutes after injection of medetomidine and romifidine in this study without significant difference. However, Figueiredo et al., (2005) demonstrated that romifidine produced a significant increase of the latency time to hoof withdrawal in horses administered IV with romifidine. In agreement with our results, the results of Kinjavdekar et al. (2006) indicated romifidine to be able to produce a mild to moderate degree of hindquarter analgesia after subarachnoid administration in goats. They added that the mechanism of analgesic action of subarachnoidally administered romifidine was probably due to the stimulation of α2 adrenoceptors at the spinal cord level. Molina and Herrero (2006) described also that the analgesic effectiveness of medetomidine, is either because its main place of action is supraspinal or because the effect is a result of an interaction of medetomidine-mediated antinociceptive (reducing sensitivity to painful stimuli) activity at spinal and supraspinal sites.

Although there was a significant increase in serum glucose in both groups as compared to the baseline values, this increase was higher in group II after injection of romifidine than that in group I after injection of medetomidine. Lyons (1997) explained that analgesia and anesthesia are usually followed by increased cortisone level, which subsequently suppresses insulin release leading to increase in serum glucose level. Group II, in this study, showed a significant increase in serum urea level at 15, 30, 60 and 90 minutes after romifidine injection while group I showed mild non-significant increase. This result suggests that romifidine produced a pronounced effect on the kidney function than did medetomidine. Serum AST and LDH showed significant increases in both groups all over the study period. CPK increased significantly in group I at 30, 60 and 90 minutes after medetomidine injection while greater significant increase was shown in group II all over the study period. These results were consistent with that of Raskalli et al. (1990) who
stated that the marked changes in the function of cardiac muscle following detomidine (related to medetomidine) injection are decrease in heart rate, cellular injury, and ultrastructural changes in the key subcellular organelles mediated in the form of leakage of cardiac enzymes (AST, LDH, and CPK).

On the basis of the results of the present study it could be concluded that medetomidine at the dose rate of 10 µg/kg and romifidine at the dose rate of 50 µg/kg produced only moderate degree of sedation, and mild degree of muscle relaxation and analgesia in buffalo calves. These effects are considered beneficial for safe investigation and clinical examination in these animals. Further restraint or conjunction with other local or general anesthetic is needed for surgical approaches. Medetomidine is considered superior to romifidine because the later induces prolonged cardiopulmonary depression which may be alarming, and also it induces comparatively higher serum levels of urea and cardiac enzymes.

REFERENCES


