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Research article

Clinical, behavioral, hematological and serum biochemical effects of neostigmine in normal dairy cows following subcutaneous and intramuscular administration of single dose

Zuhair Bani Ismail[1]*, Amin Al-Zghoul[1] and Iyad Al-Zoubi[2]

[1]Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid 22110, Jordan; [2]Private veterinarian and farm owner, Irbid 22110, Jordan

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Abstract

The objectives of this study were to describe the clinical, behavioral, hematological and serum biochemical effects of neostigmine following subcutaneous and intramuscular administration to normal dairy cows. A total of 8 normal, non-pregnant dairy cows were used in 2 experiments with 1 week rest time between the experiments. In the first experiment, cows received a single injection of neostigmine (0.02mg/kg) subcutaneously. In the second experiment, cows received a single injection of neostigmine (0.02mg/kg) intramuscularly. Cows were closely monitored for any clinical or behavioral effects before (T0), and at T10, T20, T30 and T60 following drug administration. Whole blood was collected from all cows before (T0) and after drug administration at T30 and T60. In both experiments, there were a significant increase ($P<0.05$) in the frequency of rumen motility and a significant decrease in systolic blood pressure. Cows developed signs of mild sedation (score 1 out of 3) for the entire observation period in both experiments. The frequency of bowel movement was significantly increased ($P<0.05$) with the passage of large volume of semi-liquid fecal material. Although, there was no atxia or recumbency, all cows exhibited mild signs of abdominal pain. There were no significant changes in any of the other clinical, hematology or serum biochemistry parameters. In conclusion, both subcutaneous and intramuscular administration of a single injection of neostigmine at a dose rate of 0.02mg/kg resulted in increased rumen motility and frequency of bowel movement in normal dairy cows.

Keywords: gastro-intestinal dysfunction; ruminants; bowel movement; prokinetic drugs

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Introduction

Gastro-intestinal dysfunction is a major cause of illness in lactating dairy cows especially in the immediate postpartum period (Mulligan and Doherty, 2008; Mulligan and Doherty, 2008). Hypocalcemia, endotoxemia, alkalemia, hyperglycemia, increased luminal osmolality and high energy diets play important physiological roles in impairing gastro-intestinal function in ruminants (Nouri and Constable, 2006).

***Corresponding author:** Zuhair Bani Ismail, Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid 22110, Jordan, E-mail: zuhair72@just.edu.jo

Clinically, cows suffering from such disorders have poor appetite, do not ruminate and suffer substantial loss in milk production. Such cows are also at an increased risk of developing other important metabolic diseases such as displacement of the abomasum, ketosis, fatty liver and reproductive inefficiencies (Nouri and Constable, 2006).

Many prokinetic drugs have been used to stimulate, coordinate, and restore gastro-intestinal function including abomasal, pyloric, and small intestinal motility in dairy cattle with variable clinical outcome (Nouri and Constable, 2006, Sen et al., 2006). Neostigmine is one of the most commonly administered drugs to dairy cattle that are suspected of having gastro-intestinal disorders (Sen et al., 2006). Neostigmine is a parasympatho mimetic drug that indirectly stimulates both nicotinic and muscarinic receptors (Emirleroglu et al., 2011). The exact mechanism of action of neostigmine is through a reversible inhibition of the enzyme acetylcholinesterase in tissues leading to the interference of acetylcholine breakdown (Emirleroglu et al., 2011).

In dairy cow medicine, there are no clinical trials that critically evaluate the clinical efficacy and safety of neostigmine in normal dairy cows. Therefore, the objectives of this study were to describe the clinical, behavioral, hematological and serum biochemical effects of neostigmine following subcutaneous and intramuscular administration to normal dairy cows.

Materials and Methods

All the experimental procedures performed in this study were reviewed and approved by the institutional Animal Care and Use Committee of Jordan University of Science and Technology (JUST-ACUC). A total of 8 adult cows (4-6 years of age) with an average body weight of 565±17kg were used in the study. Cows were selected randomly from one herd located in Northern Jordan. Selected cows were subjected to a complete physical examination to ensure they were healthy before the start of the study.

Two experiments were conducted using the same 8 cows with 1 week rest period between the experiments. In the first experiment, cows received a single injection of neostigmine methylsulfate (Prostigmin, Valeant Pharmaceuticals International, Inc., Switzerland) at 0.02mg/kg subcutaneously. In the second experiment, each cow received a single injection of neostigmine at 0.02mg/kg intramuscularly.

Cows were closely monitored for any clinical or behavioral effects before (T0) and after administration of the drug at 10 min (T10), 20 min (T20), 30 min (T30) and 60 min (T60). The following clinical parameters were recorded: the heart rate (beats/min), respiration rate (breaths/min), rectal temperature (C^o) using a digital thermometer, rumen motility frequency (per 3 min), strength and characterization, and systolic and diastolic blood pressure from the base of the tail using Omron digital blood pressure device (Omron Health Care, The Netherlands). This devise was previously validated for use in animals in our clinic.

Behavioral observations were consistent of evaluating the general demeanor of the cow (depression, agitation, signs of pain or distress). These parameters were assessed as present or absent. If present, the degree was assessed as mild, moderate or severe. Each cow was then assessed for the presence or absence of sedation and ataxia (DeRossi et al., 2010, Atiba et al., 2015). The degree of sedation was given a score of 0 to 3, where 0 means no sedative effects, 1 means mild sedation (sight lowering of the head carriage and or prostration of the lower lip), 2 means moderate sedation (signs of mild sedation plus presence of prolapsed third eyelid and ptyalism), 3 means severe sedation (signs of moderate sedation plus the animal leans on stanchions for support) (DeRossi et al 2010, Atiba et al 2015). Ataxia was assessed using a scale of 0 to 3 where 0 means no ataxia (walks without staggering), 1 means mild ataxia (slight stumbling but easily able to continue walking), 2 means moderate ataxia (marked stumbling), and 3 means severe ataxia (unable to stand and falling) (DeRossi et al 2010, Atiba et al 2015). Defecation and urination were assessed by recording the number of bowel movement during the entire observation period (60 min). The consistency and volume of the fecal material were also noted.

Whole blood samples were collected via coccygeal venipuncture using vacutainer needle and vacutainer tubes from each cow before (T0) and at 30 min (T30) and 60 min (T60) after drug administration. At least 10 ml of blood were collected and placed in EDTA-containing and plain blood collection tubes for hematology and serum biochemical analyses using previously published methods (Thrall et al 2004). The hematology analyses included determination of the total white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) using an electronic cell counter (Scil Vet ABC Hematology Analyzer, Scil Animal Care Company, USA).

For the serum biochemical analyses, serum was obtained by allowing samples to clot at room temperature and then centrifuged at 3000g for 10 min. The serum was then analyzed to determine total protein (TP), albumin, blood urea nitrogen (BUN), creatinine, aspartate transaminase (AST), alanintransferase (ALT), alkaline phosphatase (ALP), calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), and chloride (Cl) using previously published methods (Thrall et al 2004).

Statistical analysis

Data are presented as means ± standard deviation (Mean ± SD). In order to determine whether there was any significant difference between various data obtained in the 2 experiments before and after the treatment, data were analyzed by two way repeated measures analysis of variance (ANOVA) followed by the post hoc using the Bonferroni test. All statistical analyses were performed using SAS 9.3 Statistical Software Package. Values were considered significant at $P<0.05$.

Results

All cows tolerated the injection of neostigmine by both subcutaneous and intramuscular routes at a dose rate of 0.02mg/kg. The various clinical and behavioural parameters in dairy cows after subcutaneous and intramuscular administration of neostigmine are presented in Table 1-4. In both experiments, there were a significant increase ($P<0.05$) in the frequency of rumen motility and a significant decrease in systolic blood pressure. In cows that received neostigmine subcutaneously, rumen motility increased from 2 contractions per 3 minutes to 3 and 4 contractions per 3 minutes at T10 and T30 respectively and remained at 4 contractions per 3 minutes for the remaining of the observation time. Gastro-intestinal sounds were characterized as borborygmi- like sound.

The frequency of bowel movement was significantly increased ($P<0.05$) with the passage of large volume of semi-liquid fecal material in both experiments. Cows passed large amounts of semi-liquid feces twice within the experimental observation period starting at T20 and T30 in subcutaneous and intramuscular groups respectively.

The systolic blood pressure was significantly decreased in both experiments. In the subcutaneous group, the mean systolic blood pressure was 146 mmHg at T0. This value decreased gradually thereafter reaching a value of 120 mmHg at T60. The mean systolic blood pressure in the intramuscular group at T0 was 143mmHg. This value also decreased gradually after drug administration reaching 120mmHg at T60. The diastolic pressure decreased throughout the observation period but not statistically significant.

Table 1: Clinical parameters (mean ± SD) in dairy cows before and after subcutaneous administration of a single dose of neostigmine (n=8)

Parameter	Observation time (min)					
	T0	T10	T20	T30	T45	T60
Heart rate (beats/min)	50±4	50±4	54±4	50±4	50±4	50±4
Respiration rate (breaths/min)	30±3	32±3	32±3	32±3	32±3	32±3
Rectal temperature (C°)	38.7±0.1	38.9±0.1	38.8±0.1	38.7±0.1	38.6±0.1	38.6±0.1
Rumen motility (/3 min)	2±0.5^a	3±0.7^b	3±0.5^b	4±0.8^b	4±0.7^b	4±0.8^b
Systolic blood pressure (mmHg)	146±10^a	139±8^a	133±9^b	124±6^b	123±5^b	120±5^b
Diastolic blood pressure (mmHg)	50±3	60±6	50±5	60±4	50±4	50±3

Mean values bearing different superscripts in a row differ significantly ($P<0.05$).

Table 2: Behavioral observations in dairy cows before and after subcutaneous administration of a single dose of neostigmine (n=8)

Parameter	Observation time (min)					
	T0	T10	T20	T30	T45	T60
Sedation score	0	0	1	1	1	1
Ataxia score	0	0	0	0	0	0
Salivation	Absent	Absent	Present	Present	Present	Present
Agitation	Absent	Absent	Absent	Absent	Absent	Absent
Defecation	None	None	Yes	None	Yes	None
Urination	None	Yes	None	None	None	Yes
Laying down	None	None	None	None	None	None

Table 3: Clinical parameters (mean ± SD) in dairy cows before and after intramuscular administration of a single dose of neostigmine (n=8)

Parameter	Observation times (min)					
	T0	T10	T20	T30	T45	T60
Heart rate (beats/min)	46±6	48±7	46±4	56±8	60±9	64±8
Respiration rate (breaths/minute)	32±4	32±3	36±6	32±3	36±4	32±3
Rectal temperature (C°)	38.6±0.1	38.6±0.1	38.6±0.1	38.5±0.1	38.6±0.1	38.6±0.1
Rumen motility (/3 min)	2±0.5^a	4±0.7^b	4±0.5^b	4±0.8^b	4±0.7^b	4±0.8^b
Systolic blood pressure (mmHg)	143±7^a	134±8^a	123±3^b	120±2^b	121±3^b	120±3^b
Diastolic blood pressure (mmHg)	67±2	60±3	60±5	60±5	60±5	60±5

Mean values bearing different superscripts in a row differ significantly ($P<0.05$).

Table 4: Behavioral observations in dairy cows before and after intramuscular administration of a single dose of neostigmine (n=8)

Parameter	Observation time (min)					
	T0	T10	T20	T30	T45	T60
Sedation score	0	1	1	1	1	1
Ataxia score	0	0	0	0	0	0
Salivation	Absent	Present	Present	Present	Present	Present
Agitation	Absent	Absent	Absent	Absent	Absent	Absent
Defecation	None	None	None	Yes	None	Yes
Urination	None	Yes	None	None	None	None
Laying down	None	None	None	None	None	None

In both experiments, cows developed signs of mild sedation (score 1 out of 3) for the entire observation period. Although, in the intramuscular group, sedation became evident faster (T0) compared to T20 in the subcutaneous group. Sedation was evident by lowered head carriage and increased salivation in both groups.

Although, there was no ataxia or recumbency, all cows exhibited mild signs of abdominal pain. This was exhibited by frequent shifting of weight on the hind limbs. There were no significant changes in any of the other clinical, hematology or serum biochemistry parameters.

Discussion

There is a complex and clinically important relationship between gastro-intestinal motility and disease development in ruminants (El-Khodery and Sato, 2008). Therefore, proper understanding of the pathogenesis and causative relationships in particular clinical cases is essential for proper management and successful outcome. Although commonly used in clinical practices, there is no proven scientific evidence that neostigmine alters or improves gastro-intestinal functions or motility in either normal or ill cows (El-Khodery and Sato, 2008). Therefore, in this study, the effects of administering neostigmine to normal dairy cows was evaluated using 2 different administration routes, subcutaneously and intramuscularly using recommended dose rate of 0.02mg/kg.

In this study, the number of rumen contractions was significantly increased after neostigmine administration to normal cows using either the subcutaneous or intramuscular route indicating possible beneficial effect. In addition, the frequency, consistency and volume of bowel movement in treated cows were noticeably different compared to control cows. These results are contradictory to those reported previously (Wittek and Constable, 2005, El-Khodery and Sato, 2008). It was found that neostigmine did not significantly increase reticular contractions rate and strength or alter abomasal emptying rates. However, in the lower gastro-intestinal tract, neostigmine was reported to significantly increase the cecocolic motility patterns (Steiner and Roussel, 1995). Moreover, subcutaneously administered neostigmine was found to improve cecal function in cows with cecal dilatation (El-Khodery and Sato, 2008). In this study, the gastro-intestinal sounds were characterized as borborygmi-like which may indicate increased large intestinal motility and functions. This also may explain the consistency and volume of the passed fecal material after neostigmine administration.

In this study, there was a significant decrease in systolic blood pressure in treated cows. It is well known that the vagal influences on the heart are increased by the administration of anticholinesterases such as neostigmine (Kaya et al., 2004). This effect is expressed as a decreased in the effective refractory period of atrial muscle and an increased in the refractory period and conduction time at the sino-atrial (SA) and atrio-ventricular (AV) nodes. Therefore, the predominant effect on the heart is bradycardia caused by the accumulation of acetylcholine and thus a decrease in cardiac output and blood pressure (Kaya et al., 2004).

In this study, cows appeared mildly sedated after the administration of neostigmine by both routes as evidenced by lowered head carriage and increased salivation. In human medicine, it has been found that neostigmine administered epidurally exerts a non-dose dependent sedation in women undergoing caesarean section (Nelson et al., 1999). This effect has not been reported in cows and further studies are warranted to understand its mechanism and potential applications in post-operative pain management.

Side effects associated with neostigmine administration were reported previously. In human medicine, intrathecal neostigmine administration was associated with severe nausea, vomiting, and abdominal cramp (D'Angelo et al., 2001, Khalaf, 2015). Similar to the previous findings, cows in this study exhibited signs of mild abdominal pain most likely due to intestinal spasms and increased peristaltic movements.

In cattle, atrial fibrillation was reported in 3 cows that were treated using neostigmine for gastro-intestinal dysfunction requiring surgical intervention (Constable et al., 1990). This side effect was not reported in this study. In fact, the heart rate and rhythm remained unchanged during the entire observation period in the study.

Conclusion

Both subcutaneous and intramuscular administration of a single injection of neostigmine at a dose of 0.02mg/kg resulted in increased rumen motility and frequency of bowel movement in normal dairy cows. However, clinical assessment of neostigmine as a prokinetic drug in cows suffering from gastrointestinal dysfunction is still warranted.

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