Haryana Vet. (June, 2014) **53 (1)**, 58-62

Research Article

EVALUATION OF DIAZEPAM-KETAMINE AS ANAESTHETIC COMBINATION IN BUFFALO CALVES

AMRESH KUMAR, ASHOK KUMAR*, SUKHBIR SINGH and RAM NAZAR CHAUDHARY
Department of Veterinary Surgery and Radiology, College of Veterinary Sciences
Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125 004

Received: 02.04.2014; Accepted: 29.06.2014

ABSTRACT

A study was undertaken on six male buffalo calves by administering diazepam (0.75 mg/kg, IV) and ketamine (6.0 mg/kg, IV) 10 min after diazepam. All the animals went into sternal recumbency immediately after diazepam administration followed by lateral recumbency at 6.89 min. Relaxation of muscles was excellent. Swallowing reflex was abolished. There was complete analgesia at fetlock, base of tail, abdomen, ribs' periosteum and base of horn and it remained for 21.47 min. Recovery was manifested by the movement of limbs at 50.32 min with return of head righting reflex at 54.25 min. Complete recovery took 142.36 min (114 to 164 min). Rectal temperature was significantly lower during anaesthesia. Heart rate remained elevated throughout the entire period of observation. A significant increase in aspartate transaminase and alanine transaminase was observed in these calves. Bilirubin remained significantly elevated during anaesthesia. Magnesium was significantly lower at 24 hour after recovery.

Key words: Diazepam, ketamine, buffalo calves

Diazepam has been widely used in veterinary anaesthesia which produces dose dependent sedation and hypnosis. The intramuscular administration of diazepam in calves does not produce desired sedation, rather produces muscle rigidity, whereas, intravenous administration (0.4 mg/kg) produces sedation of 6 to 12 min along with muscle relaxation (Mirakhur *et al*., 1984). Singh and Kumar (1988) observed tranquillization and good muscle relaxation in goats with fair degree of analgesia following intravenous administration of diazepam (0.5 mg/kg). Diazepam produced minimal cardiovascular and respiratory effects in healthy horses (Muir *et al*., 1982). Diazepam (0.5 mg/kg, IV) produced hypotension without compensatory tachycardia in buffaloes (Singh *et al*., 2007). The combination of diazepam and ketamine has been reported to decrease the tidal volume with a rise in respiratory rate and diazepam also nullified the hypertensive action of ketamine in crossbred male cow calves (Singh *et al*., 1991a). Ketamine has been reported to increase heart rate and produce anaesthesia and analgesia of short duration when given alone or after chlorpromazine medication in buffalo calves (Pathak *et al*., 1982). The combination of diazepam-ketamine has not been used in buffaloes, hence the study was planned with the objective to evaluate the efficacy and safety of diazepam–ketamine anaesthetic combination in buffalo calves.

*Corresponding author: professorashokkumar@gmail.com

MATERIALS AND METHODS

The study was undertaken on six male buffalo calves of 6 to 12 months of age and weighing between 105 and 135 kg. Diazepam @ 0.75 mg/kg was injected intravenously in sternal recumbency and 10 min later ketamine was injected @ 6.0 mg/kg intravenously. Various parameters were studied before administration of drugs and at different time intervals after drug administration. The parameters were behavioural characteristics, rectal temperature (RT), heart rate (HR), respiratory rate (RR), haemoglobin (Hb), packed cell volume (PCV), plasma glucose, urea nitrogen, creatinine, total plasma proteins, albumin, inorganic phosphorus, calcium, magnesium, sodium, potassium, chloride, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase and bilirubin. Blood samples were collected via jugular venipuncture before administration of the diazepam, 10 min after administration of diazepam, 5 min after administration of ketamine, at complete recovery and at 24 hour after recovery. The data was statistically analyzed by one-way-analysis of variance and Duncan's multiple range test (Duncan, 1955). The study was undertaken after

due approval from the Institutional Animal Ethical Committee of the university.

RESULTS AND DISCUSSION

The effects of administration of diazepam-ketamine combination on behavioural parameters are shown in Table 1. All the animals went into sternal recumbency immediately after intravenous administration of diazepam with drooping of lower lip at 1.41 min and lowering of head with chin on ground at 2.40 min of diazepam administration. The animals appeared sleepy with eyes completely closed at 5.05 min. Animals went into lateral recumbency at 6.89 min of diazepam administration. The depression of CNS is mediated through depression of limbic system and due to enhancement of γ-amino butyric acid (GABA) by binding of diazepam to $GABA_A$ receptor, an inhibitory channel which when activated, decreases neuronal activity (Hall *et al.,* 2001). The similar observations were also reported with down time of 30 sec after detomidine-diazepam-ketamine administration in buffalo calves (Pawde *et al.,* 2000) and the weak time of 1.72 min after diazepam administration in atropinized goats (Chitale *et al.,* 1999). There was copious watery salivation (5.89 min) and slight drop-wise lacrimation (7.36 min). A marked salivation has been reported in calves when diazepam was administered alone (Kumar *et al.,* 2006). There was complete relaxation of muscles of tail, anus, prepuce, neck, limbs, jaw and tongue. The relaxation of muscle was due to inhibition of internuncial neurons at spinal level by diazepam (Hall *et al.,* 2001) which was also observed by Mirakhur *et al.* (1989) and Nain *et al.* (2010). Swallowing reflex was abolished. Ketamine was administered 10 min after diazepam. Palpebral reflex was abolished at 16.54 min. The reflexes were abolished due to the CNS depression produced by diazepam. Similar findings in crossbred male cow calves (Singh *et al.,* 1991b) and in dogs (Kumar *et al.,* 1990) have also been observed. There was complete analgesia at fetlock, base of tail, abdomen, ribs' periosteum and base of horn and it remained for 21.47 min in this study. The analgesia was mediated by the antagonistic effects of ketamine on N-methyl-D-aspartate (NMDA) receptor (Kohrs and Durieux, 1998). Sarma and Kumar (1998) observed surgical anaesthesia of 33.75 min in crossbred male cow calves and Kumar *et al.* (1990) reported surgical anaesthesia of 38.25 min in dogs after atropine-diazepam-ketamine anaesthesia. Recovery was manifested by the movement of limbs at 50.32 min with return of head righting reflex at 54.25 min (Table 1). All the animals returned to sternal recumbency at 61.33 min but kept the head tugged in the flank. Animals started nibbling grass at 75.34 min. All animals took 2-3 attempts to stand and stood up at 91.50 min with hind limbs held apart and head down (Table 1). Complete recovery took 142.36 min (114 to 164 min). Similar observations have also been observed by Kumar *et al.* (2006). Complete recovery has been reported to take place in 127.75 min in buffalo (Pawde *et al.,* 2000) and 113.20 min in dogs (Kumar *et al.,* 1990) after detomidine-diazepam-ketamine anaesthesia.

Table 1
Different behavioural characteristics induced by the administration of diazepam-ketamine combination in buffalo calves

Reflexes		Mean time (Min)±SE
Drooping of lower lip		1.41±0.14
Eyes closure		5.05±0.57
Onset of salivation		5.89±0.73
Assumptions lateral recumbency		6.89±1.18
Onset lacrimation		7.36±0.62
Loss of palpebral reflex		16.54±2.44
Relaxation of muscle	Prepuce	9.03±2.31
	Tail	10.30±2.04
	Neck	2.40±0.76
	Limbs	10.52±1.98
	Jaw	21.51±1.10
Tongue reflex	Loss	20.69±0.73
	Regain	49.07±3.08
Swallowing reflex	Loss	23.89±1.04
	Regain	48.28±2.76
Onset of analgesia	Fetlock	22.40±1.34
	Abdomen	22.81±1.17
	Base of horn	23.65±1.01
	Ribs	26.62±1.45
Loss of analgesia	Ribs	39.72±1.42
	Base of horn	43.37±1.80
	Abdomen	42.95±1.70
	Fetlock	43.95±1.83
Regaining of muscle tone		50.32±2.77
Eyes open		52.67±2.16
Regaining of head righting reflex		54.25±3.89
Return to sternal recumbency		61.33±6.08
Browsing time		75.34±7.89
Standing with ataxia		91.50±9.28
Complete recovery without ataxia		142.36±7.38

The effects of diazepam-ketamine combination on rectal temperature, heart rate, respiratory rate, Hb and PCV are shown in Table 2. Rectal temperature was significantly lower at 5 min (36.62°C), at 15 min of ketamine administration (36.53°C), and at complete recovery (36.95°C) in comparison to base value (38.45°C). Reduced rectal temperature was probably due to reduced basal metabolic rate, decreased muscle activities leading to production of less heat in the body and depression of hypothalamic thermoregulatory centre of brain (Ponder and Clark, 1980; Kandpal and Kumar, 1998). Decrease in rectal temperature was also recorded in atropinized goats following diazepam-ketamine and romifidine-ketamine anaesthesia (Chitale *et al.*, 1998). There was no significant variation in respiratory rate during the period of observation, however, heart rate increased significantly (60.67/min) at 5 min of ketamine administration which was earlier lowered by diazepam (47.17/min). Heart rate remained elevated throughout the study period. Increased heart rate might be due to cardiovascular stimulant property of ketamine, which is due to action on sympathetic trunk and inhibition of neuronal uptake of catecholamine by sympathetic nerve endings (Tweed *et al.*, 1972; Ivankovitch *et al.*, 1974) or may be due to increase in central release of catecholamine resulting in tachycardia (Hardie and Lukasik, 2007). The intravenous administration of diazepam followed by ketamine increased the heart rate as well as cardiac output with little change in blood pressure in dogs (Haskins *et al.*, 1986). Kumar *et al.* (1990) also reported the increased heart rate after ketamine administration in dogs. A non-significant decrease in Hb and PCV was observed during anaesthesia. Similar findings in bovine pediatric patients after atropine-ketamine and atropine-diazepam-ketamine anaesthesia (Kandpal and Kumar 1998) and with atropine-diazepam and atropine-diazepam-ketamine anaesthesia (Chitale *et al.*, 1998) have been reported. A significant decrease in Hb and PCV in goats after induction of ketamine with and without diazepam and triflupromazine (Kumar *et al.*, 1985) and after diazepam administration with and without atropine (Singh and Kumar, 1987) has earlier been reported. The decreased Hb and PCV may be attributed to sequestration of erythrocyte into spleen and extravascular compartment or haemodilution caused by water shift (Kandpal and Kumar, 1998).

The effects of diazepam-ketamine combination on plasma biochemical parameters are shown in Table 3. There was a non-significant hyperglycaemia at 5 min of ketamine administration which became normal at recovery. The hyperglycaemia may be due to release of catecholamine in a stressful condition during anaesthesia resulting in glycogenolysis (Tammisto *et al.*, 1982) or due to decreased glucose utilization, impaired insulin activity or increased adrenocortical hormone (Kumar *et al.*, 1989). Kandpal and Kumar (1998) reported a significant increase in glucose level after atropine-ketamine and non-significant increase after atropine-diazepam-ketamine anaesthesia in bovine pediatric patient. Kumar and Singh (1979) also reported an increase in glucose level one hour after ketamine-xylazine anaesthesia in bovine pediatric surgery. Hyperglycaemia has been reported to occur at maximum depth of anaesthesia after diazepam-xylazine-ketamine induction in cow calves (More *et al.*, 1993). A significant increase in ALT in this study was observed at 10 min of diazepam administration and at recovery. A significant increase in AST was also observed at recovery. The increase in liver serum marker enzymes is mainly due

Table 2
Effects of diazepam-ketamine anaesthesia on clinical and haematological parameters in buffalo calves*

Parameters (units)	Before administration of drugs	At 10 min of diazepam administration	At 5 min of ketamine administration	At recovery	At 24 hours of recovery
Ambient temperature (^{0}C)	24.80^{a}±2.14	25.08^{a}±2.15	25.50^{a}±2.09	27.70^{a}±1.85	28.67^{a}±1.43
Rectal temperature (^{0}C)	38.45^{c}±0.41	37.65^{abc}±0.45	36.62^{a}±0.55	36.95^{ab}±0.25	37.95^{bc}±0.24
Heart rate (beats/min)	51.67^{ab}±2.6	47.17^{a}±2.6	60.67^{b}±2.4	58.50^{ab}±3.5	57.83^{ab}±3.4
Respiration rate (per min.)	15.33^{a}±1.69	15.67^{a}±1.38	14.50^{a}±2.46	15.50^{a}±1.36	16.50^{a}±1.09
Haemoglobin (g/dl)	11.67^{a}±0.45	11.00^{a}±0.29	10.92^{a}±0.27	11.22^{a}±0.29	11.23^{a}±0.31
Packed cell volume (%)	27.17^{a}±0.91	26.50^{a}±0.92	25.83^{a}±0.70	26.08^{a}±0.92	24.50^{a}±0.92

*Means with different superscripts in a row differ significantly ($p<0.05$)

Table 3
Effects of diazepam-ketamine anaesthesia on certain biochemical parameters in buffalo calves*

Parameters (units)	Before administration of drugs	At 10 min of diazepam administration	At 5 min of ketamine administration	At recovery	At 24 hours of recovery
Plasma glucose (mg/dl)	73.05^{ab}±4.77	75.37^{ab}±2.79	107.07^{b}±21.57	71.4^{a}±7.27	75.07^{ab}±6.73
BUN (mg/dl)	30.67^{a}±9.40	31.88^{a}±10.37	30.93^{a}±9.16	31.63^{a}±10.39	39.28^{a}±12.51
Creatinine (mg/dl)	2.27^{a}±0.14	2.62^{a}±0.10	2.50^{a}±0.12	2.55^{a}±0.13	2.28^{a}±0.12
Total proteins (g/dl)	8.05^{a}±0.43	8.62^{a}±0.61	8.99^{a}±0.88	8.78^{a}±0.72	7.77^{a}±0.48
Albumin (g/dl)	4.24^{a}±0.22	4.81^{a}±0.32	4.99^{a}±0.49	4.68^{a}±0.41	4.31^{a}±0.30
Globulin (g/dl)	3.98^{a}±0.29	3.81^{a}±0.31	4.00^{a}±0.40	4.10^{a}±0.34	3.63^{a}±0.25
Albumin:globulin ratio	1.12^{a}±0.03	1.27^{b}±0.05	1.25^{ab}±0.04	1.14^{ab}±0.06	1.15^{ab}±0.06
Calcium (mg/dl)	10.17^{a}±1.38	10.22^{a}±0.55	9.82^{a}±0.95	10.28^{a}±1.69	9.40^{a}±0.77
Phosphorus (mg/dl)	11.57^{a}±1.03	9.57^{a}±0.80	10.90^{a}±1.03	10.60^{a}±0.88	12.13^{a}±0.94
Magnesium (mEq/L)	4.56^{bc}±0.15	4.34^{bc}±0.41	4.99^{c}±0.70	3.72^{ab}±0.31	2.95^{a}±0.16
Chloride (mEq/L)	89.72^{a}±2.31	97.56^{a}±1.77	96.65^{a}±3.09	95.08^{a}±4.19	90.45^{a}±0.65
Sodium (mmol/L)	141.70^{a}±1.92	140.48^{a}±2.98	138.25^{a}±3.17	141.22^{a}±3.82	142.12^{a}±2.67
Potassium (mmol/L)	6.12^{a}±0.31	5.70^{a}±0.39	6.18^{a}±0.33	5.67^{a}±0.66	6.72^{a}±0.83
ALT (U/L)	44.14^{a}±2.05	51.67^{b}±2.10	48.36^{ab}±2.15	51.80^{b}±1.62	47.76^{ab}±2.77
AST (U/L)	146.35^{a}±7.60	167.47^{ab}±5.56	158.62^{ab}±6.66	172.70^{b}±5.89	157.12^{ab}±7.87
ALKP (U/L)	89.00^{a}±11.92	65.33^{a}±9.30	59.67^{a}±7.41	67.83^{a}±11.42	93.67^{a}±12.32
Bilirubin (mg/dl)	0.07^{a}±0.02	0.47^{b}±0.12	0.46^{b}±0.09	0.41^{b}±0.09	0.14^{a}±0.03

*Means with different superscripts in a row differ significantly ($p<0.05$)

to leakage of these enzymes from liver cytosol into the blood stream as a result of oxidative tissue damage induced by diazepam (Abdelmajeed, 2009) or may be due to increased permeability of AST and ALT through plasma membrane of hepatic cells in diazepam-ketamine anaesthetized animals which might have occurred as a result of oxidative transformation of these drugs in the liver during the process of elimination (Vikers *et al.*, 1984). An increase in the activity of these enzymes has been reported after diazepam-chloral hydrate anaesthesia in crossbred male cow calves by Bains *et al.* (1991) and a significant rise in ALT was also reported by Pandey and Sharma (1994) in canine after diazepam-ketamine anaesthesia. Elevated activity of these two liver enzymes was also reported by Kumar *et al.* (1989) during maximal depth of anaesthesia in dogs. In this study, bilirubin remained significantly elevated during anaesthesia *i.e.* at 10 min of diazepam administration, at 5 min of ketamine administration and at complete recovery. Total bilirubin in blood is an indicator of liver function as well as erythrocyte status of the body. Its level rises because of increased production (as in haemolysis), decreased clearance, inadequate conjugation, or impaired biliary excretion (Rothuizen, 2000). Chitale *et al.* (1999) reported non-significant rise in bilirubin.The magnesium level was significantly lower at 24 h after recovery in comparison to the base value. This low magnesium was within the normal physiological range having no clinical significance. Underfeeding or fasting also lowers plasma Mg concentration. Therefore, any condition associated with a reduction in feed intake may result in lowering of Mg (Capen and Rosol, 1989). There were no significant variations in other blood biochemical parameters. On the basis of the findings of the study, it was concluded that diazepam-ketamine combination produced anaesthesia of duration of 37.72 min with least adverse effects on cardiopulmonary and haematobiochemical parameters.

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