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Research Article

EVALUATION OF ROCURONIUM AS NEUROMUSCULAR BLOCKING AGENT IN BUFFALOES

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ABSTRACT

In present study, the effect of rocuronium (0.2 mg/kg body weight intravenously) on behavioral, physiological, haematological and biochemical parameters was studied in five healthy male buffaloes of eight to twenty-four months of age. All animals showed reduced muscle activities with ataxia at 0.53± 0.02 minute and became laterally recumbent at 0.98± 0.03 minute of rocuronium administration. Relaxation of jaw muscles was observed at 4.07±0.41 min. and intubation was done at 6.52± 0.16 minutes. Complete recovery occurred in 31.22± 0.76 minutes of rouronium administration. There was a significant increase in heart rate which remained high till ten minutes of rocuronium administration. The respiration rate decreased five minutes after administration of rocuronium with lowest rate of respiration observed at fifteen minutes of rocuronium administration. There was a significant increase in haemoglobin, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, total platelet count, plasma glucose, potassium, triglycerides, ALT and AST at five minutes of rocuronium administration. It was concluded that rocuronium (0.2 mg/kg body weight intravenously) produces an effective, quick and safe, medium duration of neuromuscular block in buffaloes.

Keywords: Buffaloes, Neuromuscular block, Rocuronium.

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Neuromuscular blocking agents interfere with the effectiveness of the endogenous neurotransmitter acetylcholine to activate nicotinic cholinergic receptors of skeletal muscle cells, thereby inhibiting receptor–coupled trans-membrane ion movements necessary for muscle contraction (Bouzat *et al.*, 2004 and Unwin, 2005) resulting in skeletal muscle relaxation (Kita and Goodkin, 2000). Neuromuscular blocking agents are mostly used as adjunct to anaesthesia for reducing the dose of anaesthetics, to facilitate tracheal intubation, orthopaedic manipulations and prevention of patient movement during delicate ocular, neurological, or cardiac surgery (Taylor, 2006). Rocuronium (Org 9426), a steroidal non-depolarizing neuromuscular blocker, is a derivative of vecuronium. It has a rapid onset of action, but potency is about one-fifth of the vecuronium with some mild vagolytic activity and cardiovascular side effects (Muir *et al.*, 1989; Cason *et al.*, 1990; Marshall *et al.*, 1994).

Due to scarcity of research on muscle relaxants in buffaloes, the present study was designed to assess the efficacy and safety of rocuronium in buffaloes.

MATERIALS AND METHODS

Five experimental trials were conducted in apparently healthy male buffaloes of eight to twenty-four months of age. All animals were maintained on standard ration and under similar managemental conditions. Pilot trials were conducted to standardize the dose and route of administration of rocuronium. Based on the results of pilot trials, the dose rate of rocuronium was chosen as 0.2 mg/kg body weight intravenously. Animals were kept off feed for 24 hours and off water for 12 hours prior to experiment. The evaluation of the effects of rocuronium was based on recording of following parameters:

Behavioural changes, rectal temperature, heart rate, respiratory rate, haemoglobin, packed cell volume (PCV), total erythrocyte count (TEC), total leucocyte count (TLC), total platelet count, lymphocyte count, monocyte count, granulocyte count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), plasma glucose, urea nitrogen, creatinine, bilirubin, cortisol, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, total plasma proteins, albumin, alanine amino transferase (ALT), aspartate amino transferase (AST), lactate dehydrogenase (LDH), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), calcium, phosphorus, sodium, potassium and chloride. Recording of rectal temperature, ambient temperature, heart rate and respiratory rate was done before drug administration, at 5, 10, 15, 30, 45 min of administration of drug, at recovery and at 24 hours of recovery.

Blood samples were collected from jugular venipuncture before drug administration, at five minute of

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injection, at recovery and at 24 hours of recovery were used for haematological and biochemical studies. Haematological parameters were estimated in automatic analyzer MS4 after collecting blood samples in vials containing EDTA. Blood samples for analysis of biochemical parameters were collected in two sets of test tubes. One set of test tubes containing 3.8% sodium fluoride solution (10 mg/ml of blood) for estimation of glucose and other set containing heparin (10 units/ml) for estimation of remaining parameters as mentioned earlier. Plasma was harvested by centrifugation at 3000 rpm for 20 minutes and then stored at -20 °C. Biochemical parameters were analysed with automatic analyzer using commercially available standard system pack kits. Sodium, potassium and chloride were analyzed with automatic electrolyte analyzer. The statistical analysis of data was done by one-way-analysis of variance and Duncan's multiple range test (Duncan, 1955).The experiments were conducted with prior permission of the institutional animal ethical committee.

RESULTS AND DISCUSSION

The effects of administration of rocuronium in Mean±SE (in minutes) on behavioral parameters and body reflexes are shown in Table 1 and Table 2. All animals showed ataxia at 0.53± 0.02 minute and became laterally recumbent at 0.98± 0.03 minute of rocuronium administration. This may be due to anticholinergic action of neuromuscular blocking agents at nicotinic receptors of the skeletal muscle cells. It inhibits the receptor coupled movement of ions across the cell membrane and thereby inhibiting muscle contractions (Bouzat *et al.*, 2004 and Unwin, 2005). Muir *et al.*, (1989) concluded that rocuronium block the neuromuscular transmission by non depolarising mechanism. The onset of action was reported to be fast also in cat and pig (Muir *et al.*, 1989), dogs (Dugdale *et al.*, 2002) and in horses (Auer and Moens, 2011).

The fore limbs (2.30± 0.37 min.) were relaxed earlier than the hind limbs (3.01± 0.24 min.) while regaining of reflexes occurred earlier in hind limb (20.00±1.95 min.) followed by forelimb (21.58± 1.65 min.). Relaxation of jaw muscles was observed at 4.07±0.41 min. and intubation was done at 6.52±0.16 min. The early signs of recovery i.e. spontaneous movement of tail were noticed at 18.09±1.66 min. of rocuronium administration. Sternal recumbency was regained at 26.98± 0.64 min. The complete recovery without ataxia from neuromuscular block occurred 31.22± 0.76 min. after rocuronium administration which was in consonance with findings of Auer and Moens (2011) in horses and Auer (2007) in dogs at two different doses.

Table 1

Effect of rocuronium on different behavioural parameters in male buffaloes during the experiment

Parameters	Mean ±SE (Min.)
Weak time	0.53 ± 0.02
Down time	0.62± 0.02
Chin on ground	0.67± 0.04
Turning of neck	0.73± 0.03
Lateral recumbency	0.98± 0.03
Paddling of limbs	2.63± 0.39
Salivation	3.56± 0.14
Mouth gag application	4.54± 0.18
Intubation	6.52± 0.16
Extubation time	22.78± 0.68
Return to sternal recumbency	26.98± 0.64
Standing with ataxia	28.42± 0.60
Walking with ataxia	29.00± 0.63
Walking without ataxia	31.22± 0.76

Table 2

Effect of rocuronium on loss and gain of different body reflexes in male buffaloes during the experiment

Reflex	Loss Mean ± SE (Min.)	Gain Mean ± SE (Min.)
Tongue Reflex	4.37 ± 0.55	13.88 ± 0.42
Swallowing Reflex	5.75 ± 0.35	19.33 ± 0.50
Jaw Tone	4.07 ± 0.41	23.08± 0.60
Tail Tone	2.54± 0.36	18.09 ± 1.66
Forelimb Tone	2.30± 0.37	21.58 ± 1.65
Hind limb Tone	3.01± 0.24	20.00 ± 1.95
Prepuce Tone	7.18 ± 0.36	20.44 ± 0.73
Scrotal Tone	7.19± 0.35	22.11 ± 1.31

The effects of rocuronium on rectal temperature, heart rate and respiratory rate as Mean±SE are shown in Table 3. There was a significant increase in heart rate at 5 min. (89.80±3.77 beats/min.) of rocuronium administration as compared to the base value (51.6±1.89 beats/min.). A statistically significant increase in heart rate had been reported after rocuronium administration by Wierda *et al.* (1997). Rocuronium has minimum cardiovascular effects but in anesthetized cats it causes mild vagolytic effect and tachycardia (Marshall *et al.*, 1994). In most of the studies, it was concluded that rocuronium was devoid of adverse cardiovascular effects despite of causing mild tachycardia which was also in consonance with the present study (Cason *et al.*, 1990).

There was a significant decrease in respiratory rate at 5 min. of rocuronium administration (12.2±1.16 breaths/min.), which remained significant lower till 45 minutes

Table 3

Effects of rocuronium administration on rectal temperature, heart rate and respiratory rate in male buffaloes

Parameters (Units)	Before drug admn.	At 5 min.	At 10 min.	At 15 min.	At 30 min.	At 45 min.	At recovery	At 24 hrs. of Recovery
Ambient temperature (°C)	16^{a} ±0.40	16.00^{a} ±0.40	16.00^{a} ±0.40	16.00^{a} ±0.40	16.30^{a} ±0.34	16.34^{a} ± 0.4	16.40^{a}±0.43	17.02^{a} ±0.5
Rectal temperature (°C)	37.46^{a} ±0.35	37.12^{a} ±0.48	37.36^{a} ±0.45	37.06^{a} ±0.47	37.14^{a} ±0.24	37.12^{a} ±0.19	37.24^{a} ±0.26	37.6^{a} ±0.17
Heart rate (beats/min.)	51.6^{a} ±1.89	89.8^{b} ±3.77	76^{b} ±4.37	60.4^{a} ±3.33	54.4^{a} ±2.01	51^{a} ±1.30	51.2^{a}±1.07	51.6^{a} ±1.81
Respiratory rate (breaths/min.)	22.6^{c} ±1.21	12.2^{ab} ±1.16	10^{ab} ±0.84	7.6^{a} ±0.93	10.2^{ab} ±0.97	11.6^{ab} ±1.21	14.4^{bc} ±0.93	20.2^{c} ±0.67

Means with different superscripts vary significantly ($p<0.05$) within rows

Table 4

Effects of rocuronium administration on haematological parameters in male buffaloes

Parameters (Units)	Before Drug admn.	At 5 Min	At recovery	At 24 hrs. of recovery
Haemoglobin (g/dl)	9.28^{a} ±0.38	11.12^{b} ±0.52	9.00^{a} ±0.69	9.42^{a} ±0.40
Packed cell volume (%)	34.60^{a} ±1.18	34.64^{a} ±1.22	32.14^{a} ±0.75	34.78^{a} ±0.98
TEC ($x10^{6}$ /mm^{3})	7.17^{ab} ±0.21	7.60^{b} ±0.38	6.42^{a} ±0.23	7.40^{b} ±0.25
TLC ($x10^{3}$/mm^{3})	9.15^{a} ±0.53	9.66^{a} ±1.09	9.01^{a} ±0.85	9.26^{a} ±0.70
Total platelets count ($x10^{3}$/mm^{3})	221.40^{b} ±12.33	239.00^{c} ±13.13	241.00^{c} ±13.94	204.80^{a} ±10.78
Lymphocytes (%)	52.34^{a} ±2.46	52.22^{a} ±2.27	57.24^{a} ±1.52	52.88^{a} ±2.40
Monocytes (%)	2.00^{a} ±0.08	1.96^{a} ±0.07	2.12^{a} ±0.07	1.98^{a} ±0.11
Granulocytes (%)	45.66^{a} ±2.38	45.82^{a} ±2.25	40.64^{a} ±1.56	45.14^{a} ±2.36
MCV (fl)	51.98^{a} ±0.99	52.08^{a} ±0.84	51.70^{a} ±0.93	52.24^{a} ±0.95
MCH (pg)	14.70^{a} ±0.34	14.22^{a} ±0.36	14.90^{a} ±0.31	14.96^{a} ±0.20
MCHC (%)	29.30^{a} ±1.54	30.86^{b} ±1.54	30.62^{b} ±1.38	29.56^{a} ±1.36

Means with different superscripts vary significantly ($p<0.05$) within rows.

(11.6±1.21) of rocuronium administration with a minimum value (7.60±0.93 breaths/min.) at 15 minutes of rocuronium as compared to the base value (22.6±1.21 breaths/min.). Kumar *et al.* (2013) reported that the change in respiration rate was as low as 9.66±0.21 after administration of rocuronium in dogs. Balaji (2008) reported low respiration rate after rocuronium administration in dogs.

The effects of rocuronium on haematological parameters (Mean±SE) are shown in Table 4. There was significant increase in haemoglobin (11.12±0.52 g/dl) and MCHC (30.86±1.54%) at 5 min where MCHC remain high till recovery as compared to their respective base values (9.28±0.38 g/dl and 29.30±1.54%, respectively). Increase in haemoglobin may be due to hypoxic conditions induced by lowered respiration rate. Erythropoietin hormone is secreted in response to systemic hypoxia resulting in compensatory increase in haemoglobin (Weiss and Wardrop, 2010). Dhankhar *et al.* (2020) reported similar results using vecuronium in buffaloes. The total platelet counts increased significantly at five minutes and remained high till recovery but decreased significantly at twenty four hours of recovery. The values remained within normal physiological limits (Brar *et al.*, 2000).

The effects of rocuronium on plasma biochemical parameters (Mean±SE) are shown in Table 5. There was a significant increase in glucose (128.51±6.57mg/dl) and potassium (6.43±0.34 mmol/L) at 5 min. of rocuronium administration as compared to their respective base values (61.90±1.65 mg/dl and 5.45±0.40 mmol/L). The increase in cortisol level may be the reason for high blood sugar (Toshihiko *et al.*, 1996). Cortisol level remained non-significantly higher till recovery which may be due to the release of ACTH from the anterior pituitary gland due to hypoxic condition (Brunt and Ganong, 1963).

The significant increase in potassium level at 5 minutes may be due to hypoxia causing decrease in concentration of intracellular ATP in skeletal muscles leading to opening of ATP-sensitive K+ channel (Castle

Table 5

Effects of rocuronium on biochemical parameters in male buffaloes

Parameters (Units)	Before Drug admn.	At 5 min.	At recovery	At 24 hrs. of recovery
Glucose (mg/dL)	$64.90^{a} \pm 4.65$	$128.54^{c} \pm 6.57$	$109.46^{a} \pm 4.60$	$70.60^{a} \pm 4.12$
BUN (mg/dL)	$27.62^{b} \pm 1.54$	$29.62^{c} \pm 1.17$	$28.74^{b} \pm 1.24$	$26.74^{a} \pm 1.47$
Creatinine (mg/dL)	$1.00^{a} \pm 0.21$	$1.16^{ab} \pm 0.19$	$1.08^{b} \pm 0.20$	$1.11^{ab} \pm 0.18$
Total Bilirubin (mg/dl)	$0.18^{a} \pm 0.03$	$0.18^{a} \pm 0.03$	$0.21^{a} \pm 0.04$	$0.18^{a} \pm 0.03$
Bilirubin Direct (mg/dL)	$0.12^{a} \pm 0.01$	$0.13^{a} \pm 0.02$	$0.14^{a} \pm 0.03$	$0.13^{a} \pm 0.02$
Indirect Bilirubin (mg/dl)	$0.06^{a} \pm 0.01$	$0.05^{a} \pm 0.02$	$0.07^{a} \pm 0.03$	$0.06^{a} \pm 0.02$
Cortisol (ng/ml)	$27.73^{a} \pm 1.37$	$31.68^{a} \pm 3.50$	$29.37^{a} \pm 1.16$	$27.89^{a} \pm 0.70$
Total cholesterol (mg/dL)	$62.20^{a} \pm 2.60$	$65.40^{a} \pm 3.93$	$64.80^{a} \pm 4.35$	$64.00^{a} \pm 2.35$
HDLC (mg/dL)	$43.70^{a} \pm 3.27$	$43.34^{a} \pm 3.08$	$39.72^{a} \pm 2.88$	$41.66^{a} \pm 2.95$
LDLC (mg/dL)	$13.00^{a} \pm 1.54$	$16.56^{a} \pm 3.85$	$19.58^{a} \pm 4.85$	$16.84^{a} \pm 1.63$
Triglycerides	$12.38^{a} \pm 1.41$	$15.94^{b} \pm 1.06$	$17.58^{c} \pm 1.05$	$12.57^{a} \pm 1.46$
Total protein (g/dL)	$7.14^{ac} \pm 0.41$	$6.62^{b} \pm 0.32$	$6.43^{ab} \pm 0.33$	$7.45^{c} \pm 0.41$
Albumin (g/dL)	$2.88^{a} \pm 0.19$	$3.09^{a} \pm 0.27$	$2.98^{a} \pm 0.24$	$3.09^{a} \pm 0.20$
Globulin (g/dL)	$4.26^{a} \pm 0.34$	$3.53^{b} \pm 0.17$	$3.45^{ab} \pm 0.14$	$4.36^{ab} \pm 0.36$
A:G ratio	$0.69^{a} \pm 0.06$	$0.88^{a} \pm 0.08$	$0.86^{a} \pm 0.06$	$0.73^{a} \pm 0.08$
ALT/SGPT (IU/L)	$29.58^{a} \pm 1.49$	$33.54^{c} \pm 1.72$	$31.66^{b} \pm 1.39$	$29.60^{ab} \pm 1.35$
AST/SGOT (IU/L)	$45.30^{a} \pm 3.56$	$47.92^{b} \pm 3.48$	$45.78^{a} \pm 2.88$	$44.40^{a} \pm 3.79$
LDH (IU/L)	$536.80^{a} \pm 58.14$	$530.00^{a} \pm 54.13$	$525.60^{a} \pm 48.95$	$528.80^{a} \pm 54.63$
GGT (IU/L)	$24.11^{a} \pm 1.71$	$25.28^{a} \pm 1.69$	$24.57^{a} \pm 1.80$	$23.22^{a} \pm 1.75$
ALP (IU/L)	$94.80^{a} \pm 10.86$	$84.20^{a} \pm 11.32$	$81.80^{a} \pm 9.72$	$90.20^{a} \pm 7.36$
Calcium (mg/dL)	$10.23^{a} \pm 0.43$	$11.44^{a} \pm 0.59$	$10.84^{a} \pm 0.41$	$10.57^{a} \pm 0.43$
Phosphorus (mg/dL)	$5.55^{a} \pm 0.34$	$6.40^{a} \pm 0.19$	$6.33^{a} \pm 0.40$	$5.73^{a} \pm 0.07$
Sodium (mmol/L)	$143.12^{a} \pm 3.37$	$141.84^{a} \pm 3.67$	$144.02^{a} \pm 2.20$	$138.30^{a} \pm 2.90$
Potassium (mmol/L)	$5.45^{a} \pm 0.40$	$6.43^{b} \pm 0.34$	$5.50^{a} \pm 0.20$	$5.01^{a} \pm 0.21$
Chloride (mmol/L)	$101.98^{a} \pm 3.44$	$105.72^{a} \pm 4.07$	$101.08^{a} \pm 3.55$	$98.36^{a} \pm 2.47$

Means with different superscripts vary significantly ($p<0.05$) within rows.

and Haylett, 1987). In conditions of acidemia resulting from hypoventilation hydrogen ions enter the intracellular compartment in exchange with potassium ions. Therefore, the potassium ion level increases in plasma (Reece *et al.*, 2015). Similar findings have been reported by Dhankhar *et al.* (2020). There was a significant increase in triglycerides at 5 min. (15.94±1.06 mg/dl) of rocuronium administration and remained high till recovery as compared to the base value (12.38±1.41 mg/dl). This might be due to hypoxic conditions as explained earlier. There was a significant decrease in total protein (6.62±0.32 g/dL) and globulin (3.53±0.12 g/dL) at 5 min as compared to their respective base values (7.14±0.41 g/dL and 4.26±0.34 g/dL). There was a significant increase in ALT (33.54±1.72 IU/L) and AST (47.92±3.48 IU/L) at 5 min of rocuronium administration as compared to their respective base values (29.58±1.49 IU/L and 45.30±3.56 IU/L). The level of ALT remained significantly high till recovery (31.66±1.39 IU/L). The increased level of ALT indicates hepatocellular or myocytic injury but does not specify any cause. In dogs and cats, the ALT activity is four times greater in liver than other organs but also there is considerable activity in heart and skeletal muscles (Keller, 1981).

CONCLUSION

It can be concluded from the present study that rocuronium at a dose rate of 0.2 mg/kg body weight intravenously is an effective, safe, rapid and medium duration neuromuscular blocking agent in buffaloes.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest regarding the present research work.

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