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Article

Anaesthetic Efficacies of Epidurally Administered Lignocaine-HCl and Bupivacaine-HCl Alone and Their Combination in Rabbits

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Abstract: The present study aimed to evaluate the anaesthetic efficacy of epidurally administered lignocaine-HCl and bupivacaine-HCl, both individually and in combinations, in rabbits. A total of 15 rabbits was equally divided into three groups. Group A received an epidural injection of 4 mg/kg of 2% lignocaine-HCl, while Group B received 1 mg/kg of 0.5% bupivacaine-HCl. Group C was administered a combined solution of 2 mg/kg of 2% lignocaine-HCl and 0.5 mg/kg of 0.5% bupivacaine-HCl. Physiological parameters such as heart rate, respiratory rate, and rectal temperature were recorded 10 minutes before and then at 10-minute intervals for 120 minutes post-epidural anaesthesia. Additionally, the onset and duration of anaesthesia, onset and duration of loss of weight-bearing ability, and onset and duration of flaccid paralysis were observed following epidural administration. The anaesthesia onset times were 8.0 ± 0.354 minutes, 12.5 ± 0.224 minutes, and 10.1 ± 0.272 minutes in rabbits receiving lignocaine, bupivacaine, and the lignocaine + bupivacaine combination, respectively. The duration of anaesthesia was significantly higher ($P < 0.01$) in Group B (138.00 ± 5.15 minutes) than in Group A (50.60 ± 1.60 minutes) and Group C (87.20 ± 5.05 minutes). The onset and duration of loss of weight-bearing ability and flaccid paralysis were significantly higher ($P < 0.05$) in Group B (17.60 ± 0.245 minutes) compared to Groups A and C. The lumbosacral epidural administration of the combined lignocaine and bupivacaine solution provided enhanced anaesthetic effects compared to lignocaine or bupivacaine alone.

Keywords: Analgesia; Regional anesthetics; Lumbosacral; Rabbits

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1. Introduction

Lumbosacral regional anesthesia results in effective analgesia due to its proximity to the spinal cord receptors responsible for the regulation and transmission of nociceptive signals [1], [2]. The epidural administration of regional anesthetic is relatively safe and provides effective anesthesia and post-operative analgesia. The drugs and their combinations have been used to induce epidural analgesia in dogs and cats [3], [4],[5]. The ideal epidural local anesthetic should have a rapid onset of action, a long duration of action, good analgesia, and muscular relaxation [6]. There is no single anesthetic that possesses all of these qualities. Although lignocaine has a short latency period for epidural anesthesia, its effectiveness becomes limited for longer surgical procedures [7]. Bupivacaine lasts longer but it takes more time to onset and its muscular relaxation is also *poor* [6]–[8]. Therefore, an agent with a rapid onset and a sufficient duration of action would be ideal and could be obtained with regional anesthetic mixtures that combine the

desirable properties of each component drug. To the knowledge of the authors, there are no studies of epidural regional anesthesia using a combination of lignocaine and bupivacaine in rabbits.

The present study aimed to compare the effects of lignocaine and bupivacaine alone and their combinations for epidural anesthesia considering the onset and duration of analgesia in rabbits.

2. Materials and Methods

Study Location

This study was done from August- 2023 to October- 2023 in the NPI college research unit

Experimental Rabbits

Fifteen clinically normal rabbits *(Oryctolagus cuniculus)* weighing 1.8 ± 2.3 kg were used in this study. They were purchased from a local rabbit market in Bharatpur. The rabbits were housed in a 12-h light-dark photoperiod in 3 different cages of the research laboratory of NPI College. They were fed seasonal grasses, concentrates, and water *ad libitum*. The rabbits were kept for 2 weeks for acclimatization in in laboratory environment. The experimental protocol was approved by the Nepal Veterinary Council on animal research (Ref. no. 35/2080/81).

Study design

Food was withheld from the rabbits for 8 hours (i.e. overnight) prior to the experiments, while water was allowed freely. The body weight of each rabbit was determined using a weighing balance. The rabbits were restrained to facilitate the epidural anesthesia. The injection site was located and the hair of the lumbosacral region was shaved and the skin was disinfected. Then after, 2% lignocaine gel was applied topically on site to reduce pain and to prevent unnecessary suffering and the epidural puncture was performed using 26 gauge*5/8" needle. A total of fifteen rabbits were divided into three groups, with each group containing five different rabbits: the LIG group, the BUP group, and the LIG+BUP group. The rabbits of LIG, BUP, and LIG+BUP groups received the lumbosacral epidural administration of 2% lignocaine (4 mg/kg) 0.5% bupivacaine (1mg/kg) and 1:1 mixture of 2% lignocaine and 0.5% bupivacaine (2 mg/kg and 0.5 mg/kg). The dose rate were used in this study following the doses of prior studies [9]. Physiological parameters were recorded 10 minutes before the epidural anesthesia and then after anesthesia at every 10 min intervals over a period of 120 min.

Assessment of Epidural Anesthesia

The onset of anesthesia was assessed by observing the reflexes to needle pinprick stimulation to the hind paddle or the web. It was performed at one-minute intervals until surgical anaesthesia. However, the duration of anesthesia was determined by the return of response to a bipedal pinprick stimulus. Loss of weight-bearing was detected when rabbits did not stand on its hind limbs. The recovery was confirmed when the rabbits became stable. Flaccid paralysis was confirmed when no measurable tone in both hind limbs. The heart rate (HR), respiratory rate (RR) and rectal temperature (RT) were recorded appropriately 10 minutes before and then after every 10 minutes interval over a period of 120 min of epidural injections of regional anesthetics.

Statistical analysis

The data are expressed as Mean ± Standard Error of Mean. The data was analyzed in Minitab version 17 software manufactured in Pennsylvania State University. Data were analyzed using one-way ANOVA followed by post hoc Tukey's test. However, the physiological variables were compared using for repeated measures in each group. The P value less than 0.05 was considered significant.

3. Results

The onset of anesthesia with LIG-Group (8.0 ± 0.354 min), BUP-Group (12.5 ± 0.224 min) and LIG-BUP combination- Group (10.1 ± 0.272 min) was significantly different (P<0.05) from each other among the three groups. The mean duration of anesthesia of LIG group (50.60 ± 1.60), BUP group (138.00 ± 5.15), and LIG+BUP group (87.20 ± 5.05) differs highly significantly (P<0.01) from each other. The mean onset of loss of weight-bearing ability was significant (P<0.05) in the BUP group with (17.6000 ± 0.245). The mean duration of loss of weight-bearing ability was highly significant (P<0.05) in the BUP group (76.80 ± 3.57) compared to LIG group and LIG+BUP which also differs from each other significantly (P<0.05) with values of (24.000 ± 0.949) and (49.00 ± 2.74). The mean onset of flaccid paresis in BUP group (19.600 ± 0.927) was highly significant (P<0.05) as compared to LIG group and LIG+BUP group with values of (23.900 ± 0.400) and (24.600 ± 0.927) respectively. The mean duration of flaccid paresis was highly significant (P<0.05) in LIG group (10.900 ± 0.332) compared to the BUP group and LIG+BUP group which also differs from each other significantly (P<0.05) with values of (50.20 ± 2.22) and (19.00 ± 1.52) respectively. There were no significant changes in heart rate, respiration rate and temperature.

Table 1: The mean ± SEM of onset time of anesthesia (min), duration of anesthesia (min), onset and duration of loss of weight bearing ability (min), onset and duration of flaccid paresis (min).

Parameter	LIG	BUP	LIG+BUP
Time of onset of anesthesia	8 ± 0.354 [a]	12.5 ± 0.224 [b]	10.1 ± 0.272 [c]
Duration of anesthetic effect	50.60 ± 1.60 [a]	138.00 ± 5.15 [b]	87.20 ± 5.05 [c]
Onset of loss of weight-bearing ability	16.00 ± 0.707 [a]	17.60 ± 0.245 [b]	16.00 ± 0.274 [a]
Duration of loss of weight bearing ability	24.00 ± 0.949 [a]	76.80 ± 3.57 [b]	49.00 ± 2.74 [c]
Onset of flaccid paralysis	23.90 ± 0.400 [a]	19.60 ± 0.927 [b]	24.60 ± 0.927 [a]
Duration of flaccid paresis	10.90 ± 0.332 [a]	50.20 ± 2.22 [b]	19.00 ± 1.52 [c]

Figures with similar symbols in the same row indicate non- significant

Table 2: The mean ± SEM of HR (beat/min) measured at different times 10 minutes prior and then after anesthesia at every 10 minute intervals over a period of 120 min post injection.

Time	LIG	BUP	LIG+BUP
-10	232.60 ± 9.79	225.4 ± 10.3	222.80 ± 4.79
10	218.60 ± 7.61	242.8 ± 15.2	215.60 ± 6.18

20	200.40 ± 8.18	213.80 ± 9.68	200.00 ± 5.67
30	208.40 ± 7.28	206.2 ± 10.8	208.40 ± 5.61
40	234.40 ± 6.76	203.2 ± 13.8	210.00 ± 7.13
50	225.2 ± 11.9	197.4 ± 12.1	208.40 ± 3.82
60	203.6 ± 12.3	204.60 ± 9.91	204.40 ± 2.99
70	203.4 ± 13.2	205.00 ± 7.63	221.60 ± 2.64
80	218.80 ± 7.71	209.80 ± 8.32	212.2 ± 10.1
90	216.40 ± 4.30	210.00 ± 6.88	218.40 ± 2.66
100	215.80 ± 4.61	203.60 ± 8.19	221.60 ± 3.06
110	219.80 ± 6.00	207.6 ± 13.7	223.20 ± 1.53
120	214.00 ± 6.03	211.4 ± 11.1	225.20 ± 2.71

Table 3. The mean ± SEM of RR (breaths/min) measured at different times 10 minutes prior and then after anesthesia at every 10 minute intervals over a period of 120 min post injection

Time	LIG	BUP	LIG+BUP
-10	129.60 ± 3.83	148.6 ± 11.8	143.2 ± 12.6
10	127.40 ± 4.49	159.6 ± 7.46	135.20 ± 6.21
20	123.40 ± 7.70	147.40 ± 7.40	141.40 ± 6.66
30	125.00 ± 7.30	138.4 ± 10.2	144.20 ± 6.33
40	122.6 ± 13.2	142.6 ± 10.9	145.20 ± 9.09
50	131.2 ± 14.6	134.0 ± 12.0	142.80 ± 8.55
60	142.4 ± 16.0	137.8 ± 12.6	149.60 ± 9.10
70	149.8 ± 15.5	136.8 ± 10.8	138.40 ± 7.09
80	140.0 ± 12.7	143.0 ± 12.3	146.60 ± 7.24
90	138.4 ± 11.0	146.6 ± 15.3	135.60 ± 7.30
100	134.20 ± 8.01	144.0 ± 17.2	135.60 ± 6.56
110	131.60 ± 9.05	146.4 ± 13.3	139.60 ± 8.42
120	129.40 ± 6.46	149.0 ± 10.8	137.00 ± 6.71

Table 4. . The mean ± SEM of RT (°F) measured at different times 10 minutes prior and then after anesthesia at every 10 minutes intervals over a period of 120 min post injection

Time	LIG	BUP	LIG+BUP
-10	101.76 ± 0.129	101.68 ± 0.0583	101.84 ± 0.211
10	101.56 ± 0.0510	101.46 ± 0.0872	101.60 ± 0.0600
20	101.54 ± 0.0510	101.32 ± 0.0735	101.42 ± 0.121
30	101.56 ± 0.103	101.40 ± 0.268	101.52 ± 0.0583
40	101.60 ± 0.0707	101.30 ± 0.103	101.36 ± 0.354
50	101.72 ± 0.116	101.34 ± 0.103	101.42 ± 0.0840

60	101.64 ± 0.0748	101.38 ± 0.0800	101.32 ± 0.371
70	101.50 ± 0.0707	101.42 ± 0.180	101.46 ± 0.0812
80	101.40 ± 0.0949	101.34 ± 0.121	101.34 ± 0.206
90	101.70 ± 0.210	101.38 ± 0.185	101.38 ± 0.273
100	101.7 ± 0.169	101.40 ± 0.0949	101.70 ± 0.0949
110	101.65 ± 0.150	101.48 ± 0.107	101.52 ± 0.0663
120	101.68 ± 0.133	101.48 ± 0.0374	101.50 ± 0.134

4. Discussion

None of the rabbits died or showed any side effects during and after anesthesia for a week period. In this study, the rabbits were gently restrained for injection of epidural anesthesia with short needles which produced no apparent discomforts. The lumbosacral epidural anesthesia technique in rabbits and ferrets *(Mustela furo)* is identical to dogs and cats, with the exception of the rarely definitive popping sensation when the intervertebral ligament is punctured [10]. The spinal cord continues caudally into the sacral vertebrae in rabbits and thereby increased the risk of puncture of both the dura and arachnoid membranes during lumbosacral epidural injection [11]. To overcome this situation, the anesthesia was administered once cerebrospinal fluid was seen in the hub of the needle. In this study, solutions of lignocaine and bupivacaine were observed to be dispersed in the syringe, showing pharmacological compatibility which is similar to previous studies [12]. The doses of LIG 0.2 ml/kg i.e. (4 mg/kg) and BUP 0.2 ml/kg i.e. (1 mg/kg) were used in this study following the doses of prior studies [9]. The combination of lignocaine and bupivacaine exhibited a longer onset of action ($P<0.05$) than lignocaine but shorter ($P<0.05$) than that of bupivacaine. This result is similar findings by [13]. Onset of action of regional anesthetics differs due to pka (acid dissociation constant or pH at which the non-ionized and ionized fractions are at equilibrium) values when pH value of tissue remains constant. Regional anesthetics with a low pKa of 7.6-7.9 have a rapid onset of action because 30% to 40% of these drugs exist in the unionized state at pH 7.4 of which lignocaine has pKa value of 7.9. Lignocaine has a faster onset time than drugs with a high pka because its pka is closer to tissue pH [7]. Conversely, regional anesthetics with high pKa of 8.0 - 8.9 are slow acting agents because only 15% or less of these drugs are unionized at pH 7.4 of which bupivacaine holds value of 8.1.

Moreover, the duration of analgesia was significantly longer with lignocaine and bupivacaine combination ($P<0.01$) than lignocaine alone and significantly shorter than bupivacaine alone ($P<0.01$), indicating that adding bupivacaine to lignocaine accelerates and prolongs the duration of anesthesia. Similar findings were reported in a previous study conducted on dogs [13]. The mean duration of loss of weight bearing ability was significantly high in lignocaine- bupivacaine combination group ($P<0.05$) than in lignocaine alone indicating that adding bupivacaine to lignocaine accelerates and prolongs the quality of analgesia. Onset of flaccid paresis was significantly rapid ($P<0.05$) in bupivacaine group. The mean duration of flaccid paresis was significantly longer in the lignocaine bupivacaine combination group ($P<0.01$) than in lignocaine alone. This finding can be attributed to a synergistic effect between lignocaine and bupivacaine. The combination of the two drugs can decrease the side effects of each drug and increase the duration of flaccid paresis and analgesia [14]. No significant differences were observed in heart rate, respiration rate and temperature between three groups.

5. Conclusions

The combination of lignocaine and bupivacaine showed an ideal anesthetic effect over lignocaine or bupivacaine alone having shorter onset and prolonged duration of action with good analgesia. None of the treatments induced significant changes in heart rate, respiration rate and temperature. Further research is needed to investigate the analgesic effect of lignocaine and bupivacaine combination in rabbit under surgical conditions.

6. References

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