Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.47750/jptcp.2023.30.04.053

Comparative effect of calcium channel blockers on the pancuronium neuromuscular blockers in Wistar rats

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Submitted: 16 January 2023; Accepted: 25 February 2023; Published: 15 March 2023

ABSTRACT

Calcium channel blockers (CCB's) are used in the treatment of cardiovascular disorders like hypertension, cardiac arrhythmias, angina, and other diseases. Several studies have reported that CCB's inhibit neuromuscular transmission. In the in vitro studies, CCB's along with standard skeletal muscle relaxants showed evidence of increasing the neuromuscular blockade effect. Neuromuscular blocking agents are used by the anaesthesiologists to improve the surgical procedures during surgery by the surgeons. Hence, in the current study, the comparative effects of verapamil, and benidipine on pancuronium neuromuscular blockers in Wistar rats were demonstrated. The 36 Wistar rats were divided into six groups, consisting of six rats in each group. The test drugs were injected intraperitoneally (IP) and neuromuscular blockade activity was observed over a period of one hour by inclined screen method and histological study of the skeletal muscles was done to compare the neuromuscular blocking effect. The comparative effect of the verapamil and benidipine indicates that it produces significant neuromuscular blocking activity and increase in the latency of hindlimb paralysis with benidipine suggest that benidipine is having more neuromuscular blocking activity when compared with verapamil.

Keywords: *hypertension, arrhythmias, neuromuscular blocker, paralysis, intraperitoneal*

INTRODUCTION

Neuromuscular blockers are commonly employed during clinical anaesthesia for improving patient compliance during endotracheal intubation, assisting mechanical ventilation, and optimising the surgical condition in the patients. It also decreases postoperative laryngeal trauma and hoarseness (1). The risk of using neuromuscular blockers in the surgery was intraoperative awareness and prolonged muscle paralysis even after the end of surgery (2).

Hence, there might be a delay in patient discharge from the post-operative care unit (3). These disadvantages made anaesthesiologists avoid the use of neuromuscular blockers in surgeries as long as the surgeon did not require it, but in many upper abdominal and laparoscopic procedures, to improve the surgical condition by paralysing the intraoperative muscles, neuromuscular blocking agents are still used (4).

CCB's are used in cardiovascular diseases like hypertension, cardiac arrythmias, angina pectoris etc. The CCB's are useful in cardiovascular disease by inhibiting the voltage gated calcium channels in the smooth and cardiac muscles results in dilation of blood vessels and improvement in the oxygen supply to the cardiac tissue (5). There is decrease in myocardial force, decrease in heart rate and conduction velocity with in the heart (6).

Excitation-contraction coupling in the skeletal muscles is dependent on the intracellular calcium which is released from the sarcoplasmic reticulum (7). The CCB's which prevent the entry of calcium in to the cells also inhibit the calcium release from the sarcoplasmic reticulum. Even though CCB's are might not produce much effect on the skeletal muscles, there are studies showed that the CCB's are inhibiting the neuromuscular transmission in anesthetized cat, dogs and in Wister rats (8). There are many studies showing the potentiation effect of CCB's with neuromuscular agents (9). However, there are no literatures for the study of comparative effect of verapamil, and benidipine on pancuronium neuromuscular blockers, hence the current study undertaken.

MATERIALS AND METHOD

Experimental animals

The male Wister rats weighing between 150-200gm were used in this study. The animal was procured and placed in animal house at standard temperature, humidity and 12 hours dark and light cycle. The food and water were supplied ad libitum. The animal experiment was approved from the Institutional Ethical Committee, Aspen Biopharma Laboratories Limited with approval number ASPEN/1018/22. All the study was conducted by following the guidelines of CCSEA, Government of India.

Test Drugs

The comparative effect of the verapamil, and benidipine was done by taking existing standard neuromuscular blocking agent pancuronium. All the drugs are obtained from the authorised dealers.

Experimental Design

A total number of 36 Wister rats are divided in to six groups each consisting of six animals in each group.

Group 1 (Control) Normal Saline 1 ml/kg.
Group 2 (Standard) Pancuronium 0.04 ml/kg.
Group 3 (Test group 1) Verapamil 5 mg/kg.
Group 4 (Test group 2) Verapamil 10 mg/kg
Group 5 (Test group 3) Benidipine 5 mg/kg
Group 6 (Test group 4) Benidipine 10 mg/kg

Procedure

The test drugs were injected by a 24–25-gauge needle attached to a 1ml syringe into the intraperitoneal cavity of rats. After injecting the drug to the animals, the neuromuscular blockade activity was observed over a period of 1 hour using inclined screen method.

Inclined screen method

The inclined screen method was used to screen the drugs which has skeletal muscle relaxant activity (10). An inclined screen was used to measure the muscle relaxant activity. The rats were placed at the upper part of the inclined screen and were given 30 seconds to hang on or to fall off.

The following parameters were observed.

1. Time of Onset of hind limb paralysis (in minutes)

2. Total duration of paralysis (in minutes)

Histological study by Haematoxylin-Eosin Staining:

For analysing histopathology, skeletal muscle tissue was administered for paraffin sectioning.

Then the tissues were hydrated and dehydrated in sorted alcohol series. It was then cleared using xylene and chloroform, and then it was fixed in paraffin wax using rotary microtome, tissues sections were taken (5µm) out and kept overnight at room temperature. It was then de-paraffinized and moistened with descending alcohol concentrations followed by dist.H2O. Using Haematoxylin and Eosin stain, the sections being stained and then administered for ascending alcohol concentrations. The permanent slide was prepared using a DPX mount. Observed the slides under a light microscope (Olympus microscope) and photomicrographs were took using a Sony digital camera (12).

Statistical analysis

Results were presented as Mean ± SD. One-way ANOVA was used for multiple comparisons followed by Dunnett's post–hoc test for comparison between groups using statistical software SPSS. For all the tests *p<0.05, **p<0.01, ***p<0.001 were considered statistically significant.

RESULTS

The onset and duration of hindlimb paralysis was recorded in minutes and given in the Table 1. The mean and standard deviation of the pancuronium (0.04mg), V\verapamil (5mg and 10mg), benidipine (5mg and 10mg) are mentioned in the Table 1.

The mean of the onset of hindlimb paralysis of the pancuronium (0.04mg), verapamil (5mg and 10mg), benidipine (5mg and 10mg) are 5.00, 12.83, 14.66, 17.83, 25.00 minutes respectively. Between the group comparison of standard and test groups showed highly statistical significance (p <0.001). Between the group comparison of normal control and test groups showed highly statistical significance (p <0.001) (Figure 1).

The mean of the duration of hindlimb paralysis of the pancuronium (0.04mg), verapamil (5mg and 10mg/kg), benidipine (5mg and 10mg) are 37.17, 12.83, 17.33, 13.16, 17.83 minutes respectively. Between the group comparison of standard and test groups showed highly statistical significance (p <0.001). Between the group comparison of normal control and test groups showed highly statistical significance (p <0.001) (Figure 2).

TABLE 1: Onset and total duration of hindlimb paralysis following the injection (in minutes)

	Normal control	Pancuronium (0.04mg/kg)	Verapamil (5mg/kg)	Verapamil (10mg/kg)	Benidipine (5mg/kg)	Benidipine (10mg/kg)
Onset of hindlimb paralysis	0.0	5.00±1.41	12.83±1.94***ab	14.66±2.16***cd	17.83±1.72***ef	25.00±2.90***gh
Duration of hind limb paralysis	0.0	37.17±3.17	12.83±1.94***ab	17.33±2.80***cd	13.16±2.13***ef	17.83±2.31***gh

The values are represented as mean ± SD. ***: p <0.001
***a, c, e, g - Values differed significantly from the standard.
***b, d, f, h - Values differed significantly from the normal group.

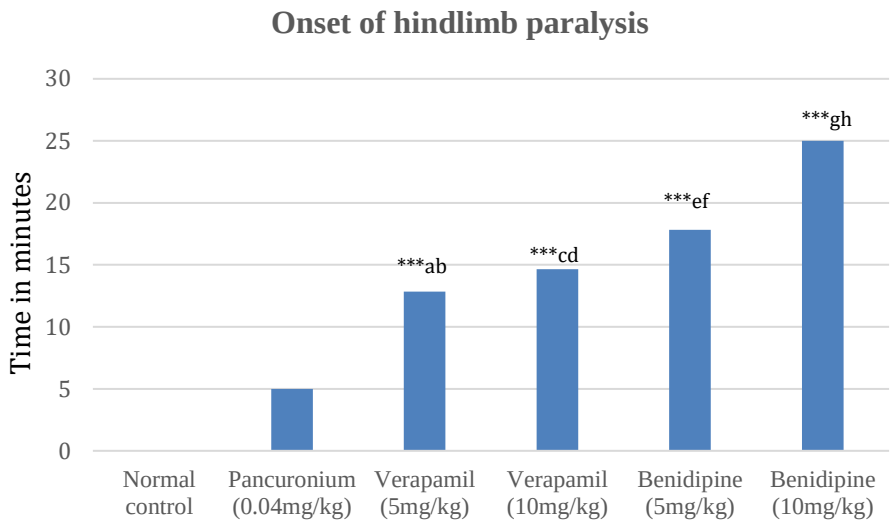

FIGURE 1: Effect of verapamil and benidipine on the onset of hindlimb paralysis in Wister rats***: p <0.001

***a, c, e, g - Values differed significantly from the standard.

***b, d, f, h - Values differed significantly from the normal control.

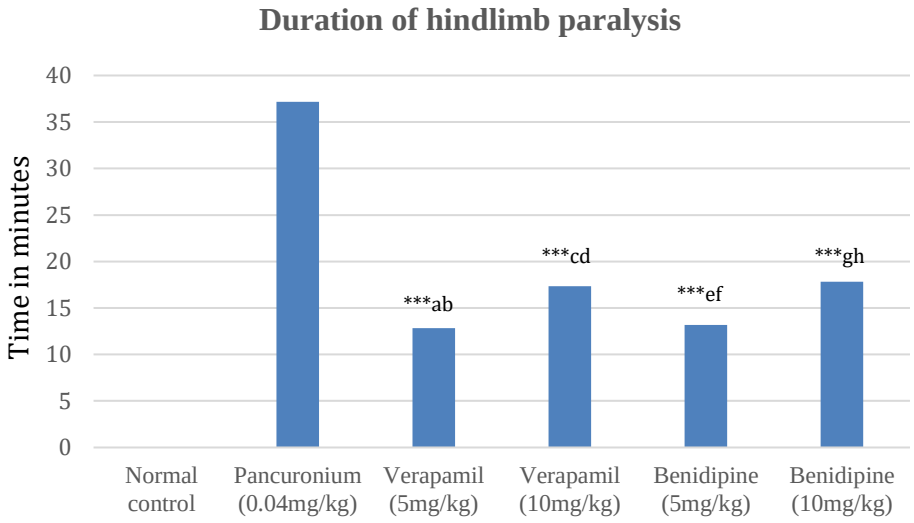

FIGURE 2: Effect of verapamil and benidipine on the duration of hindlimb paralysis in Wister rats. ***: p <0.001

***a, c, e, g - Values differed significantly from the standard.

***b, d, f, h - Values differed significantly from the normal control.

J Popul Ther Clin Pharmacol Vol 30(4):e533–e539; 15 March 2023.

Histology Study

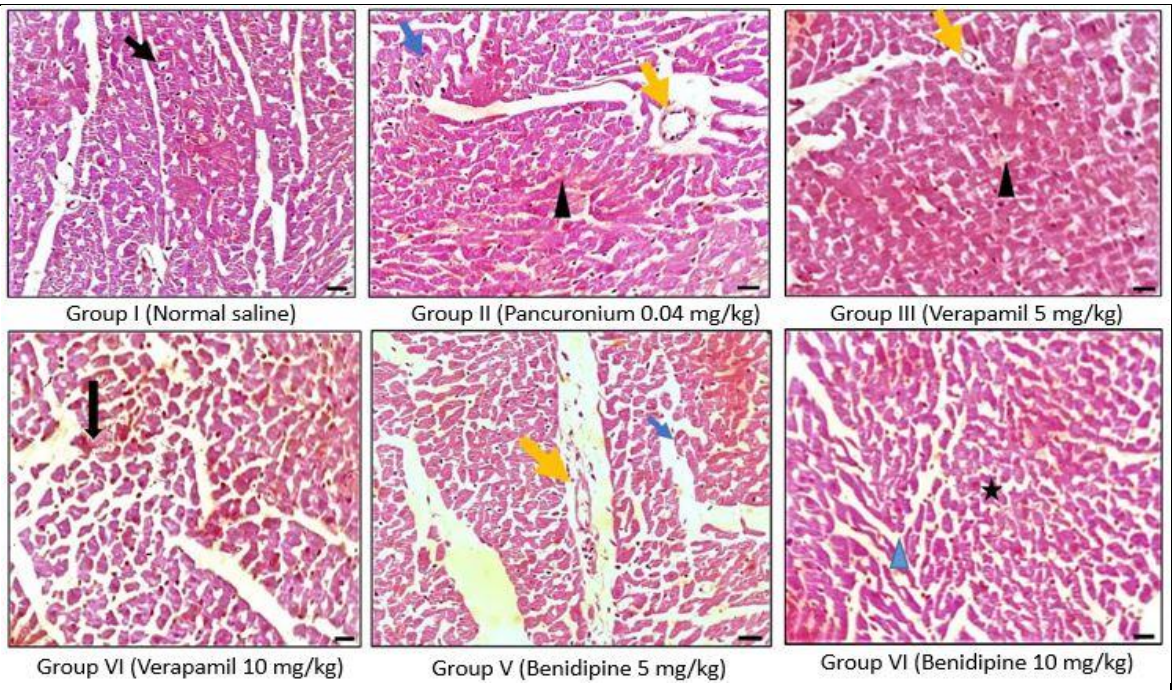

FIGURE 3: Effect of verapamil and benidipine on histology of skeletal muscle

Interpretation

Group I (Normal control): This group showed normal muscle fibres with visible cell nuclei and myofibrillar elements (black arrow)

Group II (Pancuronium 0.04 mg/kg): This group showed inflammatory infiltrates (yellow arrow), degenerated muscle fiber (arrow head) and macrophage infiltration (blue arrow).

Group III (Verapamil 5 mg/kg): This group showed less degenerated muscle fiber (arrow head) and macrophage infiltration (yellow arrow).

Group IV (Verapamil 10 mg/kg): There was irregular outer borders of the muscle fibers (broad arrow) and marked reduction in the muscle fiber size.

Group V (Benidipine 5 mg/kg): The muscle fibers exhibit macrophage infiltration (yellow arrow) and muscle oedema formation (blue arrow).

Group VI (Benidipine 10 mg/kg): There is some variability in the muscle fiber size (star) and splitting of some muscle's fibers (blue arrow head) was noticed.

DISCUSSION

The effects of CCB's are investigated in smooth muscle, cardiac muscle, respiratory muscle and for is neuromuscular blocking activity. The CCB's are more sensitivity to the voltage gated calcium channels in the cardiac muscle and smooth muscles (13). CCB's are less sensitive in skeletal muscles but binding of the drug may disrupt the mechanism coupling of electrical changes across the T membrane to calcium release from the sarcoplasmic reticulum. Due to this CCB's may affect the physiological function at the neuromuscular junction (14).

The results of the current study shown the comparative effect of the CCB's verapamil and benidipine on the pancuronium neuromuscular blocker. The neuromuscular blocking effect of two graded dose of verapamil and benidipine was compared with standard, normal control and between the test group are highly significant indicates that the test drugs affected the

neuromuscular activity at neuromuscular junction. The onset of hindlimb was increased both the test groups compared to standard. Between the test groups onset of hindlimb was more in benidipine (5mg and 10mg) when compared to verapamil (5mg and 10mg). Duration of hindlimb paralysis was significantly decreased in test groups than the standard. Between the test groups there is no significant difference in the duration of hindlimb paralysis.

There are several mechanisms are proposed to understand the mechanism of neuromuscular blocking activity of the CCB's. Calcium is essential for the release of acetylcholine at neuromuscular junction. It has been postulated that verapamil and benidipine inhibits conductance of the presynaptic membrane to calcium. Some studies suggest that verapamil and benidipine affects transmitter release or may interfere with neuromuscular transmission by blocking the action of acetylcholine (15, 16).

The administration of neuromuscular blocking agents causes increased risk of sedation, confusion and may lead to increased risk of injuries in skeletal muscles (17). Most important of these injury processes is inflammation since it is a consistent and lasting response. The inflammatory response is dependent on two factors, namely the extent of actual damage and the degree of muscle vascularization at the time of injury (18).

In the present study, there was no gross change in the cytoarchistructure of the skeletal muscle were observed. However, in drug exposed group to pancuronium (0.04mg), verapamil (5 mg, 10mg), benidipine (5mg and 10mg) showed degenerated muscle fibre, macrophage infiltration, marked reduction in muscle fibre size, splitting of some muscle fibres and muscle oedema formation (Fig 3) were noticed when compared with the control group. Because muscle contraction is largely dependent upon influx of calcium, its inhibition causes relaxation. This happens particularly in vascular and arterial smooth muscle cells resulting in arterial vasodilation. As far as the authors are aware, there are no published reports to compare with the present findings on the comparative effect of CCB's on the pancuronium more studies on the cellular activity and cytoarchistructural changes that may be responsible for this differential sensitivity need to be done.

CONCLUSION

The present study demonstrated the neuromuscular blocking activity of verapamil and benidipine. The comparative effect indicates that it produces significant neuromuscular blocking activity and increase in the latency of hindlimb paralysis with benidipine suggest that benidipine is having more neuromuscular blocking activity when compared with verapamil. Hence, clinicians should be aware of such interaction in patients receiving long term CCB's. However, further studies are warranted to develop this as a promising drug for neuromuscular blocking activity.

CONFLICTS OF INTEREST

Authors declares that there no conflicts of interest.

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